The immune divide: factors influencing immune variation and differences in vaccine responses

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THE IMMUNE DIVIDE

FACTORS INFLUENCING IMMUNE VARIATION AND DIFFERENCES IN VACCINE RESPONSES

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General introduction and thesis outline

Introduction

Vaccines represent a significant milestone in modern medicine, second only to clean water and sanitation for reducing morbidity and mortality from infectious diseases[1-3]. However, immune systems vary significantly across populations, resulting in variations in immune response to vaccines[4-8]. Understanding the factors associated with immune variability that led to differences in vaccine responses is critical to addressing poor vaccine efficacy in populations that need it the most. Leveraging advanced single-cell technologies such as Mass Cytometry (CyTOF)[9], conventional flow cytometry [10],16S rRNA Sequencing[11], other omics technologies[12-14], and advanced data analysis, researchers have been able to dissect the factors driving immune variability across different populations. These tools have also shed light on the underlying factors linked to variations in immune responses to vaccines as further demonstrated below.

Genetics, sex and age

Immune variability is partly driven by genetic background [4, 7, 15-18]. Human genetic factors such as HLA polymorphisms[19, 20], PRRs [21, 22], and cytokine production genes[21], are linked to vaccine response variability. For example, individuals with certain HLA haplotypes show higher immune responses to HIV [23] and malaria vaccines[24, 25]. Beyond genetics, sex differences significantly impacts immune variation [26, 27], with women typically generating higher antibody titers to most of the vaccines than men[28-30]. However, gender roles in low- and middle-income countries (LMICs) can confound these differences[33]. Age also modulates immune variability, especially at the extremes of life in infants[34], and the elderly due to less developed or reduced activity of the immune system[35-38]. This consequently causes poor vaccine responses in both age groups[39-42]. It is important to note that age is also influenced by extrinsic factors, including environmental influences, which play a role in immune-biological ageing.

Geographical location and seasons

Non-genetic factors often play a larger role, particularly in adaptive immunity, which is more susceptible to environmental influences[4, 7, 15-18]. Immune profiles vary between HICs and LMICs [43-45], and between rural and urban settings[44, 45]. Similarly, immunogenicity to

vaccines differs in similar patterns, for example, vaccine immunogenicity was higher in the UK and Switzerland compared to Senegal, Malawi, and Uganda[46, 47]. Higher immunogenicity to tetanus and influenza vaccines has been also observed in semi-urban compared to their rural counterparts[48, 49]. Evidence suggests that dry and wet seasons influence immunological differences[50], higher antibody levels have been observed for vaccines administered during wet seasons compared to dry seasons in some populations[51, 52].

Infections/Pathogen factors

Pathogens significantly shape immune system function, contributing to immune variation. For instance, cytomegalovirus (CMV), which infects over 90% of individuals in LMICs, is strongly linked to immune variation and impairs vaccine responses, such as those for Ebola[53-55]. Similarly, Schistosoma infection skews the immune system [44, 56] and is associated with reducing vaccine efficacy for hepatitis B[57], BCG [58], TT[59], and measles vaccines[60]. Furthermore, malaria, endemic in many tropical regions, is linked to immune variation [61-64] and is associated with lower antibody responses to vaccines like measles[65], tetanus[66], *Haemophilus influenzae* type B, *Salmonella typhi*, and *Neisseria meningitidis*[67]. Ectoparasites such as tsetse flies, kissing bugs, fleas, and ticks, can also drive immune variations through compounds they inject, though their impact on vaccines remains uncertain [68, 69]. Additionally, chronic infections such as HIV, tuberculosis, and hepatitis C virus (HCV) also contribute to immune variation and affect vaccine efficacy/immunogenicity [70].

Lifestyle and socioeconomic factors

Lifestyle factors such as smoking, exercise, sleeping and alcohol consumption are linked to immune variation[71]. Cigarette smoking is known to affect both innate- and adaptive immunity[72], leading to increased leukocytes and reduced NK cell numbers, serum immunoglobulin levels and poor vaccine efficacy/immunogenicity[73-75]. While socioeconomic status (SES) is complex and intertwined with other factors, making it difficult to isolate, low SES is linked to higher exposure to pathogens, poorer nutrition, and limited access to healthcare, all of which contribute to immune variation[76]. This, in turn, has been linked to reduced vaccine efficacy as seen with vaccines like polio [77] and oral rotavirus [78, 79]. Diet is vital for immune function, fueling both innate and adaptive systems[80, 81].

Malnutrition is linked to poor disease control and reduced vaccine responses[82]. Additionally, essential nutrients like iron and vitamin D also influence immune variation and vaccine efficacy based on their availability.

Microbiome

The microbiome significantly influences immune system variation through interactions with immune cells, affecting their development and regulatory functions[83]. Variations in microbiome composition are linked to differences in immune profiles and immune response to vaccines [84, 85]. Additionally, specific microbial populations can induce distinct immune profiles, underscoring the role of personalized microbiota in shaping immune variation[86, 87]. Although not always the case, individuals with similar microbiomes, regardless of location, tend to have comparable vaccine responses, as seen in infants from Ghana, Pakistan, and the Netherlands[88, 89]. Certain bacteria, like *Bifidobacterium longum*, enhance vaccine responses to tetanus, BCG, and Hepatitis B[90], while others, like *Proteobacteria*, are negatively associated with vaccine efficacy[40]. Factors such as delivery method at birth, diet, infections, and medications also shape microbiomes[91].

Pre-existing immunity

Pre-existing immunity can reduce vaccine efficacy. For instance, exposure to non-tuberculous mycobacteria (NTM) has been linked to lower BCG efficacy against *Mycobacterium tuberculosis*[92], similarly, exposure to Malaria has been associated with reduced or no change in antibody levels after administration of malaria vaccines[93]. In the case of yellow fever, prior vaccination can impair the boosting effect of the Yellow Fever vaccine[94], though some flaviviruses, such as dengue, benefit from prior exposure to related viruses [95]. Also, the natural infections or previous vaccinations with Ebola [96] or COVID-19[97, 98] vaccines lead to higher antibody production after subsequent vaccinations. Possibly this is due to differences in vaccine type and mechanism of action of vaccines[99].

Vaccine-related factors

Vaccine factors, such as differences in Yellow Fever vaccine strains (17D-204, 17D-213, and 17DD) used by different countries, can cause variations in vaccine efficacy/immunogenicity[100]. Dosing and schedules vary and can be linked to variations in vaccine efficacy[101][103]. Additionally, adjuvants as seen with influenza[104] and hepatitis B vaccines[105].

Baseline immune status

Finally, baseline immune status is associated with variation in vaccine response. All host factors discussed above can potentially determine the status of baseline immune status(BIS)[106]. BIS has been linked to a diversity of vaccine responses [107-110]. Baseline immune status, both innate and adaptive level, if optimized before vaccination can help improve the vaccine responses.

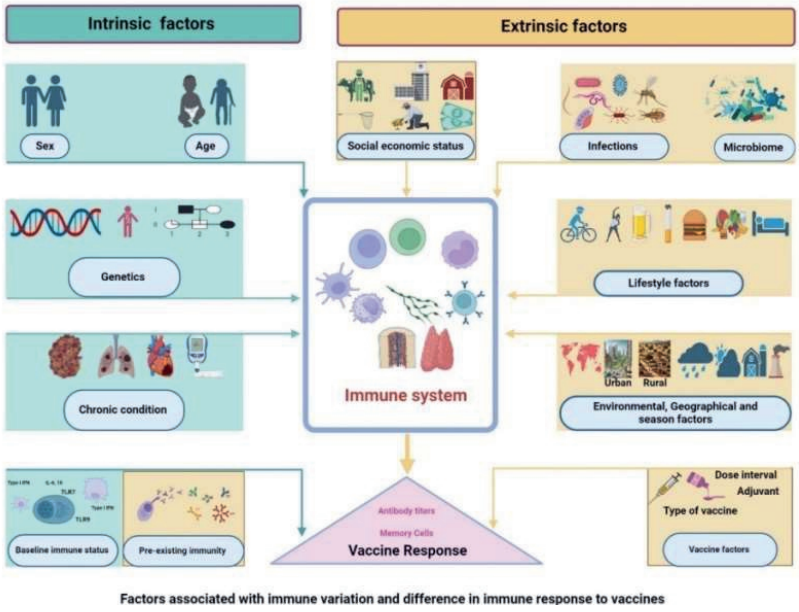

Figure 1: The factors associated with immune variation and differences in immune response to vaccines.

Potential mechanisms of pathogen-driven vaccine hypo-responsiveness

To move beyond observed associations and understand why vaccines underperform in certain populations, it is essential to explore the underlying immunological mechanisms. While this section enumerates pathogen-associated variations in vaccine efficacy, the mechanistic underpinnings such as immune exhaustion (characterized by sustained expression of PD1, TIM3, CTLA4, and diminished effector function), chronic immune activation marked by CD38+HLA-DR+ cell profiles, skewed T helper cell polarization (favoring regulatory or TH2 over TH1 responses), and structural alterations in lymphoid tissues are further dissected in **Chapter 2**. These immune dysregulations emerge in response to persistent exposure to environmental antigens, microbiome-derived metabolites, and chronic infections, particularly in low-resource settings. **Chapter 2** builds on these observations by examining how these contexts reshape the immune landscape, ultimately compromising vaccine responsiveness through exhaustion, immunosenescence, regulatory dominance, and disrupted antigen presentation.

The scope and aims of this thesis

The overarching aim of this thesis is to investigate how factors such as the microbiota, environment, lifestyle, and baseline immune profiles contribute to variations in vaccine immunogenicity. Focusing on healthy Tanzanian adults, we explore the rural–urban immune divide using high-resolution immune profiling tools such as mass cytometry, conventional flow cytometry and microbiome sequencing(16S rRNA sequencing). To achieve this, we conducted three distinct studies: two cross-sectional studies and one longitudinal cohort study. To ensure methodological rigor and minimize selection and measurement biases, all three studies employed standardized recruitment procedures, eligibility screening, and validated data collection tools. A school based approach, and community-based sensitization campaigns facilitated participant enrollment, and structured questionnaires adapted from previously validated studies used were administered by trained personnel. In the longitudinal study, participants were randomly assigned to vaccinated and control groups to reduce selection bias. In summary the first cross-sectional study focused on evaluating the prevalence and diagnostic accuracy of tools used for diagnosing schistosomiasis in a rural setting. This study involved over 500 school-aged children, providing critical insights into the prevalence and effectiveness of diagnostic methods in resource-limited environments. The second cross-sectional study aimed to compare the immunological profiles of individuals from rural and urban areas, while

identifying factors contributing to these variations. Participants were recruited from four distinct study sites, two rural and two urban, where blood, stool, and urine samples were collected. Detailed questionnaires were used to capture individual lifestyle factors such as socioeconomic status, diet, and environmental exposures, helping to elucidate the intrinsic and extrinsic drivers of immune variation.

The third study, a longitudinal cohort study, followed individuals from two of the selected study sites one rural and one urban. A total of 185 participants were recruited, with an even distribution between rural and urban settings. To examine the factors influencing vaccine response, both groups were administered the yellow fever vaccine. Biological samples (blood, stool, urine) were collected at multiple time points before vaccination, and on days 2, 7, 14, 28, 56, 90, and 178 post-vaccination. Additionally, detailed lifestyle information was gathered through questionnaires, capturing data on socio-economic factors, diet, and other relevant variables. Advanced single-cell technology, such as mass cytometry, helped in dissecting the immune cell profiles at high resolution, while 16S rRNA sequencing provided insights into microbiome composition. This integrative approach allowed for a comprehensive analysis of the intrinsic and extrinsic factors shaping immune variation and vaccine response in Tanzanian adults.

Thesis outline

This thesis is divided into seven chapters, each addressing key aspects of factors shaping immune variation and vaccine response.

The **first chapter serves** as an introduction, we provide an overview of various factors influencing immune system variation and vaccine responses, setting the foundation for this thesis.

In the **second chapter**, we conduct a comprehensive review of immunological factors linked to geographical variations in vaccine response, delving into the mechanisms behind vaccine hypo-responsiveness and global disparities in vaccine efficacy.

In the **third chapter**, we present field and laboratory-based findings on the prevalence of Schistosomiasis among school-aged children in Mwanga District, Tanzania, providing insight into the prevalence of schistosomiasis.

In the **fourth chapter**, we explore the impact of lifestyle factors on cellular immune profiles, focusing on differences between rural and urban populations in Tanzania, and analyze the factors associated with immune profile variations.

In the **fifth chapter**, we examine the association between the innate immune state at baseline and vaccine responses, aiming to gain a deeper understanding of the immunological mechanisms underlying variations in vaccine efficacy.
In the **sixth chapter**, we examine differences in gut microbiome composition between rural and urban settings and investigate the associations between gut microbiota and vaccine responses. We also compare vaccine responses between these populations.

In the **final chapter**, we discuss the key findings, synthesize the results, and propose future research directions based on the implications of the study.

Reference:

1. Greenwood, B., *The contribution of vaccination to global health: past, present and future.* Philosophical Transactions of the Royal Society B-Biological Sciences, 2014. **369**(1645).
2. Rappuoli, R., et al., *Vaccines, new opportunities for a new society.* Proceedings of the National Academy of Sciences of the United States of America, 2014. **111**(34): p. 12288-12293.
3. Rodrigues, C.M.C. and S.A. Plotkin, *Impact of Vaccines; Health, Economic and Social Perspectives.* Frontiers in Microbiology, 2020. **11**.
4. Brodin, P., et al., *Variation in the human immune system is largely driven by non-heritable influences.* Cell, 2015. **160**(1-2): p. 37-47.
5. Brodin, P., et al., *Variation in the Human Immune System Is Largely Driven by Non-Heritable Influences.* Cell, 2015. **160**(1-2): p. 37-47.
6. Souquette, A. and P.G. Thomas, *Variation in the basal immune state and implications for disease.* Elife, 2024. **13**.
7. Duffy, D., *Understanding immune variation for improved translational medicine.* Current Opinion in Immunology, 2020. **65**: p. 83-88.
8. Liston, A., E.J. Carr, and M.A. Linterman, *Shaping Variation in the Human Immune System.* Trends Immunol, 2016. **37**(10): p. 637-646.
9. Simoni, Y., et al., *Mass cytometry: a powerful tool for dissecting the immune landscape.* Current Opinion in Immunology, 2018. **51**: p. 187-196.
10. Proserpio, V. and B. Mahata, *Single-cell technologies to study the immune system.* Immunology, 2016. **147**(2): p. 133-140.
11. Johnson, J.S., et al., *Evaluation of 16S rRNA gene sequencing for species and strain-level microbiome analysis.* Nature Communications, 2019. **10**.
12. Baysoy, A., et al., *The technological landscape and applications of single-cell multi-omics.* Nature Reviews Molecular Cell Biology, 2023. **24**(10): p. 695-713.
13. Heumos, L., et al., *Best practices for single-cell analysis across modalities.* Nature Reviews Genetics, 2023. **24**(8): p. 550-572.
14. Wen, L., et al., *Single-cell technologies: From research to application.* Innovation, 2022. **3**(6).
15. Patin, E., et al., *Natural variation in the parameters of innate immune cells is preferentially driven by genetic factors (vol 19, pg 302, 2018).* Nature Immunology, 2018. **19**(6): p. 645-645.
16. Astle, W.J., et al., *The Allelic Landscape of Human Blood Cell Trait Variation and Links to Common Complex Disease.* Cell, 2016. **167**(5): p. 1415-+.
17. Farber, D.L., N.A. Yudanin, and N.P. Restifo, *Human memory T cells: generation, compartmentalization and homeostasis.* Nature Reviews Immunology, 2014. **14**(1): p. 24-35.
18. Kolaczkowska, E. and P. Kubes, *Neutrophil recruitment and function in health and inflammation.* Nature Reviews Immunology, 2013. **13**(3): p. 159-175.
19. Amirzargar, A.A., et al., *HLA-DRB1, DQA1 and DQB1 alleles and haplotypes frequencies in Iranian healthy adult responders and non-responders to recombinant hepatitis B vaccine.* Iran J Immunol, 2008. **5**(2): p. 92-9.
20. Ovsyannikova, I.G., et al., *Human leukocyte antigen and cytokine receptor*

gene polymorphisms associated with heterogeneous immune responses to mumps viral vaccine. Pediatrics, 2008. **121**(5): p. E1091-E1099.

21. Randhawa, A.K., et al., *Association of Human TLR1 and TLR6 Deficiency with Altered Immune Responses to BCG Vaccination in South African Infants.* Plos Pathogens, 2011. **7**(8).
22. Moore, C.E., et al., *Single Nucleotide Polymorphisms in the Toll-Like Receptor 3 and CD44 Genes Are Associated with Persistence of Vaccine-Induced Immunity to the Serogroup C Meningococcal Conjugate Vaccine.* Clinical and Vaccine Immunology, 2012. **19**(3): p. 295-303.
23. Gartland, A.J., et al., *Analysis of HLA A*02 association with vaccine efficacy in the RV144 HIV-1 vaccine trial.* J Virol, 2014. **88**(15): p. 8242-55.
24. Reyes, C., et al., *Critical role of HLA-DRβ* binding peptides' peripheral flanking residues in fully-protective malaria vaccine development.* Biochemical and Biophysical Research Communications, 2017. **489**(3): p. 339-345.
25. Nielsen, C.M., et al., *RTS,S malaria vaccine efficacy and immunogenicity during challenge is associated with HLA genotype.* Vaccine, 2018. **36**(12): p. 1637-1642.
26. Dunn, S.E., W.A. Perry, and S.L. Klein, *Mechanisms and consequences of sex differences in immune responses.* Nature Reviews Nephrology, 2024. **20**(1): p. 37-55.
27. Oertelt-Prigione, S., *The influence of sex and gender on the immune response.* Autoimmunity Reviews, 2012. **11**(6-7): p. A479-A485.
28. Bayas, J.M., et al., *Immunogenicity and reactogenicity of the adult tetanus-diphtheria vaccine. How many doses are necessary?* Epidemiology and Infection, 2001. **127**(3): p. 451-460.
29. Klein, S.L., A. Jedlicka, and A. Pekosz, *The Xs and Y of immune responses to viral vaccines (vol 10, pg 338, 2010).* Lancet Infectious Diseases, 2010. **10**(11): p. 740-740.
30. Hoffmann, J.P., et al., *Sex hormone signaling and regulation of immune function.* Immunity, 2023. **56**(11): p. 2472-2491.
31. Furman, D., et al., *Systems analysis of sex differences reveals an immunosuppressive role for testosterone in the response to influenza vaccination.* Proceedings of the National Academy of Sciences of the United States of America, 2014. **111**(2): p. 869-874.
32. Potluri, T., et al., *Age-associated changes in the impact of sex steroids on influenza vaccine responses in males and females (vol 4, 29, 2019).* Npj Vaccines, 2019. **4**.
33. **Drakesmith, H., et al.**, Vaccine Efficacy and Iron Deficiency: An Intertwined Pair? *The Lancet Haematology*, 2021. 8(9): p. e666–e669
34. Belnoue, E., et al., *APRIL is critical for plasmablast survival in the bone marrow and poorly expressed by early life bone marrow stromal cells.* Swiss Medical Weekly, 2008. **138**: p. 8s-8s.
35. Panda, A., et al., *Age-Associated Decrease in TLR Function in Primary Human Dendritic Cells Predicts Influenza Vaccine Response.* Journal of Immunology, 2010. **184**(5): p. 2518-2527.
36. Mogilenko, D.A., I. Shchukina, and M.N. Artyomov, *Immune ageing at single-cell*

resolution. Nature Reviews Immunology, 2022. **22**(8): p. 484-498.

37. Frasca, D. and B.B. Blomberg, *Aging induces B cell defects and decreased antibody responses to influenza infection and vaccination.* Immunity & Ageing, 2020. **17**(1).

38. Nipper, A.J., et al., *Diminished antibody response to influenza vaccination is characterized by expansion of an age-associated B-cell population with low PAX5.* Clinical Immunology, 2018. **193**: p. 80-87.

39. Falahi, S. and A. Kenarkoohi, *Host factors and vaccine efficacy: Implications for COVID-19 vaccines.* Journal of Medical Virology, 2022. **94**(4): p. 1330-1335.

40. Zimmermann, P. and N. Curtis, *The influence of the intestinal microbiome on vaccine responses.* Vaccine, 2018. **36**(30): p. 4433-4439.

41. Wolters, B., et al., *Immunogenicity of combined hepatitis A and B vaccine in elderly persons.* Vaccine, 2003. **21**(25-26): p. 3623-3628.

42. McElhaney, J.E., et al., *The unmet need in the elderly: How immunosenescence, CMV infection, co-morbidities and frailty are a challenge for the development of more effective influenza vaccines.* Vaccine, 2012. **30**(12): p. 2060-2067.

43. Labuda, L.A., et al., *A Praziquantel Treatment Study of Immune and Transcriptome Profiles in -Infected Gabonese Schoolchildren.* Journal of Infectious Diseases, 2020. **222**(12): p. 2103-2113.

44. de Ruiter, K., et al., *Helminth infections drive heterogeneity in human type 2 and regulatory cells.* Science Translational Medicine, 2020. **12**(524).

45. Mbow, M., et al., *Changes in immunological profile as a function of urbanization and lifestyle.* Immunology, 2014. **143**(4): p. 569-577.

46. Black, G.F., et al., *BCG-induced increase in interferon-gamma response to mycobacterial antigens and efficacy of BCG vaccination in Malawi and the UK: two randomised controlled studies.* Lancet, 2002. **359**(9315): p. 1393-1401.

47. Muyanja, E., et al., *Immune activation alters cellular and humoral responses to yellow fever 17D vaccine (vol 124, pg 3147, 2014).* Journal of Clinical Investigation, 2014. **124**(10): p. 4669-4669.

48. van Riet, E., et al., *Cellular and humoral responses to tetanus vaccination in gabonese children.* Vaccine, 2008. **26**(29-30): p. 3690-3695.

49. van Riet, E., et al., *Cellular and humoral responses to influenza in Gabonese children living in rural and semi-urban areas.* Journal of Infectious Diseases, 2007. **196**(11): p. 1671-1678.

50. Temba, G.S., et al., *Urban living in healthy Tanzanians is associated with an inflammatory status driven by dietary and metabolic changes.* Nature Immunology, 2021. **22**(3): p. 287-+.

51. Miles, D.J.C., et al., *Effects of antenatal and postnatal environments on CD4 T-cell responses to BCG in healthy infants in the Gambia.* Clinical and Vaccine Immunology, 2008. **15**(6): p. 995-1002.

52. Abreu, T.C., et al., *Association between season of vaccination and antibody levels against infectious diseases.* Epidemiology and Infection, 2020. **148**.

53. Griffiths, P. and M. Reeves, *Pathogenesis of human cytomegalovirus in the immunocompromised host.* Nature Reviews Microbiology, 2021. **19**(12): p. 759-773.
54. Sharpe, H.R., et al., *CMV-associated T cell and NK cell terminal differentiation does not affect immunogenicity of ChAdOx1 vaccination.* Jci Insight, 2022. **7**(6).
55. Bowyer, G., et al., *Reduced Ebola vaccine responses in CMV young adults is associated with expansion of CD57 KLRG1 T cells.* Journal of Experimental Medicine, 2020. **217**(7).
56. Gazzinelli-Guimaraes, P.H. and T.B. Nutman, *Helminth parasites and immune regulation.* F1000Res, 2018. **7**.
57. Muir, R., et al., *infection alters the host pre-vaccination environment resulting in blunted Hepatitis B vaccination immune responses.* Plos Neglected Tropical Diseases, 2023. **17**(7).
58. Elias, D., et al., *infection reduces the protective efficacy of BCG vaccination against virulent.* Vaccine, 2005. **23**(11): p. 1326-1334.
59. Sabin, E.A., et al., *Impairment of tetanus toxoid-specific Th1-like immune responses in humans infected with Schistosoma mansoni.* Journal of Infectious Diseases, 1996. **173**(1): p. 269-272.
60. Tweyongyere, R., et al., *Effect of Schistosoma mansoni infection and its treatment on antibody responses to measles catch-up immunisation in pre-school children: A randomised trial.* Plos Neglected Tropical Diseases, 2019. **13**(2).
61. de Jong, S.E., et al., *Systems analysis and controlled malaria infection in Europeans and Africans elucidate naturally acquired immunity.* Nature Immunology, 2021. **22**(5): p. 654-+.
62. Portugal, S., et al., *Malaria-associated atypical memory B cells exhibit markedly reduced B cell receptor signaling and effector function.* Elife, 2015. **4**.
63. Sundling, C., et al., *B cell profiling in malaria reveals expansion and remodeling of CD11c B cell subsets.* Jci Insight, 2019. **4**(9).
64. Weiss, G.E., et al., *Atypical Memory B Cells Are Greatly Expanded in Individuals Living in a Malaria-Endemic Area.* Journal of Immunology, 2009. **183**(3): p. 2176-2182.
65. Kizito, D., et al., *Factors affecting the infant antibody response to measles immunisation in Entebbe-Uganda.* Bmc Public Health, 2013. **13**.
66. Elliott, A.M., et al., *Effects of maternal and infant co-infections, and of maternal immunisation, on the infant response to BCG and tetanus immunisation.* Vaccine, 2010. **29**(2): p. 247-55.
67. Williamson, W.A. and B.M. Greenwood, *Impairment of the immune response to vaccination after acute malaria.* Lancet, 1978. **1**(8078): p. 1328-9.
68. Wikel, S.K., *Modulation of the host immune system by ectoparasitic arthropods - Blood-feeding and tissue-dwelling arthropods manipulate host defenses to their advantage.* Bioscience, 1999. **49**(4): p. 311-320.
69. Wilson, A.D., *Immune responses to ectoparasites of horses, with a focus on insect bite hypersensitivity.* Parasite Immunology, 2014. **36**(11): p. 558-570.
70. Vimali, J., et al., *Chronic Viral Infection Compromises the Quality of Circulating Mucosal-Associated Invariant T Cells and Follicular T Helper Cells via*

Expression of Inhibitory Receptors. Frontiers in Bioscience-Landmark, 2024. **29**(3).

71. Linfen Guo, Y.H., Jing He, Deng Li, Wei Li, Haitao Xiao, Xuewen Xu, and Y.Z.R. Wang, *Associations of lifestyle characteristics with circulating immune markers in the general population based on NHANES 1999 to 2014.* Scientifc Reports | 2024.
72. Sopori, M., *Effects of cigarette smoke on the immune system.* Nature Reviews Immunology, 2002. **2**(5): p. 372-377.
73. Ferson, M., et al., *Low Natural Killer-Cell Activity and Immunoglobulin Levels Associated with Smoking in Human-Subjects.* International Journal of Cancer, 1979. **23**(5): p. 603-609.
74. Jung, Y.S., et al., *Impact of Smoking on Human Natural Killer Cell Activity: A Large Cohort Study.* Journal of Cancer Prevention, 2020. **25**(1): p. 13-20.
75. Saint-André, V., et al., *Smoking changes adaptive immunity with persistent effects.* Nature, 2024. **626**(8000).
76. Azad, M.B., et al., *Influence of Socioeconomic Status Trajectories on Innate Immune Responsiveness in Children.* Plos One, 2012. **7**(6).
77. Grassly, N.C., et al., *New strategies for the elimination of polio from India.* Science, 2006. **314**(5802): p. 1150-1153.
78. Patel, M., et al., *Oral Rotavirus Vaccines: How Well Will They Work Where They Are Needed Most?* Journal of Infectious Diseases, 2009. **200**: p. S39-S48.
79. Zaman, K., et al., *Efficacy of pentavalent rotavirus vaccine against severe rotavirus gastroenteritis in infants in developing countries in Asia: a randomised, double-blind, placebo-controlled trial.* Lancet, 2010. **376**(9741): p. 615-623.
80. Newsholme, P., *Cellular and metabolic mechanisms of nutrient actions in immune function.* Nutrition & Diabetes, 2021. **11**(1).
81. Tourkochristou, E., C. Triantos, and A. Mouzaki, *The Influence of Nutritional Factors on Immunological Outcomes.* Frontiers in Immunology, 2021. **12**.
82. Cunningham-Rundles, S., D.F. McNeeley, and A. Moon, *Mechanisms of nutrient modulation of the immune response.* Journal of Allergy and Clinical Immunology, 2005. **115**(6): p. 1119-1128.
83. Wiertsema, S.P., et al., *The Interplay between the Gut Microbiome and the Immune System in the Context of Infectious Diseases throughout Life and the Role of Nutrition in Optimizing Treatment Strategies.* Nutrients, 2021. **13**(3).
84. Vlasova, A.N., et al., *How the gut microbiome regulates host immune responses to viral vaccines.* Current Opinion in Virology, 2019. **37**: p. 16-25.
85. Ciabattini, A., et al., *Role of the Microbiota in the Modulation of Vaccine Immune Responses.* Frontiers in Microbiology, 2019. **10**.
86. Jordan, C.K.I. and T.B. Clarke, *How does the microbiota control systemic innate immunity?* Trends in Immunology, 2024. **45**(2): p. 94-102.
87. Round, J.L. and S.K. Mazmanian, *The gut microbiota shapes intestinal immune responses during health and disease (vol 9, pg 313, 2009).* Nature Reviews Immunology, 2009. **9**(8): p. 600-600.

88. Harris, V.C., et al., *Significant Correlation Between the Infant Gut Microbiome and Rotavirus Vaccine Response in Rural Ghana.* Journal of Infectious Diseases, 2017. **215**(1): p. 34-41.
89. Harris, V., et al., *Rotavirus vaccine response correlates with the infant gut microbiota composition in Pakistan.* Gut Microbes, 2018. **9**(2): p. 93-101.
90. Huda, M.N., et al., *Bifidobacterium Abundance in Early Infancy and Vaccine Response at 2 Years of Age.* Pediatrics, 2019. **143**(2).
91. **Jeong, S.**, Factors Influencing Development of the Infant Microbiota: From Prenatal Period to Early Infancy. *Clinical and Experimental Pediatrics*, 2022. 65(9): p. 439-447.
92. Poyntz, H.C., et al., *Non-tuberculous mycobacteria have diverse effects on BCG efficacy against.* Tuberculosis, 2014. **94**(3): p. 226-237.
93. Ubillos, I., et al., *Baseline exposure, antibody subclass, and hepatitis B response differentially affect malaria protective immunity following RTS,S/AS01E vaccination in African children.* BMC Med, 2018. **16**(1): p. 197.
94. Bovay, A., et al., *Minimal immune response to booster vaccination against Yellow Fever associated with pre-existing antibodies.* Vaccine, 2020. **38**(9): p. 2172-2182.
95. Whitehead, S.S., et al., *In a randomized trial, the live attenuated tetravalent dengue vaccine TV003 is well-tolerated and highly immunogenic in subjects with flavivirus exposure prior to vaccination.* Plos Neglected Tropical Diseases, 2017. **11**(5).
96. Koch, T., et al., *Ebola Virus Disease Survivors Show More Efficient Antibody Immunity than Vaccinees Despite Similar Levels of Circulating Immunoglobulins.* Viruses-Basel, 2020. **12**(9).
97. Angyal, A., et al., *T-cell and antibody responses to first BNT162b2 vaccine dose in previously infected and SARS-CoV-2-naive UK health-care workers: a multicentre prospective cohort study.* Lancet Microbe, 2022. **3**(1): p. E21-E31.
98. Tang, J.Y., et al., *Respiratory mucosal immunity against SARS-CoV-2 after mRNA vaccination.* Science Immunology, 2022. **7**(76).
99. Vanaparthy, R., et al., *Review of COVID-19 viral vector-based vaccines and COVID-19 variants.* Infez Med, 2021. **29**(3): p. 328-338.
100. Beck, A.S. and A.D.T. Barrett, *Current status and future prospects of yellow fever vaccines.* Expert Review of Vaccines, 2015. **14**(11): p. 1479-1492.
101. Hoest, C., et al., *Evaluating Associations Between Vaccine Response and Malnutrition, Gut Function, and Enteric Infections in the MAL-ED Cohort Study: Methods and Challenges.* Clinical Infectious Diseases, 2014. **59**: p. S273-S279.
102. Abdala-Torres, T., et al., *Immune response induced by standard and fractional doses of 17DD yellow fever vaccine.* Npj Vaccines, 2024. **9**(1).
103. Amirthalingam, G., et al., *Serological responses and vaccine effectiveness for extended COVID-19 vaccine schedules in England (vol 12, 7217, 2021).* Nature Communications, 2022. **13**(1).
104. Khurana, S., et al., *MF59 Adjuvant Enhances Diversity and Affinity of Antibody-Mediated Immune Response to*

Pandemic Influenza Vaccines. Science Translational Medicine, 2011. **3**(85).

105. Jacques, P., et al., *The immunogenicity and reactogenicity profile of a candidate hepatitis B vaccine in an adult vaccine non-responder population.* Vaccine, 2002. **20**(31-32): p. 3644-3649.

106. Nehar-Belaid, D., et al., *Baseline immune states (BIS) associated with vaccine responsiveness and factors that shape the BIS.* Seminars in Immunology, 2023. **70**.

107. Hagan, T., et al., *Transcriptional atlas of the human immune response to 13 vaccines reveals a common predictor of vaccine-induced antibody responses.* Nature Immunology, 2022. **23**(12): p. 1788-+.

108. Querec, T.D., et al., *Systems biology approach predicts immunogenicity of the yellow fever vaccine in humans.* Nature Immunology, 2009. **10**(1): p. 116-125.

109. Tsang, J.S., et al., *Improving Vaccine-Induced Immunity: Can Baseline Predict Outcome?* Trends in Immunology, 2020. **41**(6): p. 457-465.

110. Odak, I., et al., *Systems biology analysis reveals distinct molecular signatures associated with immune responsiveness to the BNT162b COVID-19 vaccine.* Ebiomedicine, 2024. **99**.

Chapter 2

Immunological factors linked to geographical variation in vaccine responses

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Abstract

Vaccination is one of medicine's greatest achievements; however, its full potential is hampered by considerable variation in efficacy across populations and geographical regions. For example, attenuated malaria vaccines in high-income countries confer almost 100% protection, whereas in low-income regions these same vaccines achieve only 20–50% protection. This trend is also observed for other vaccines, such as bacillus Calmette–Guérin (BCG), rotavirus and yellow fever vaccines, in terms of either immunogenicity or efficacy. Multiple environmental factors affect vaccine responses, including pathogen exposure, microbiota composition and dietary nutrients. However, there has been variable success with interventions that target these individual factors, highlighting the need for a better understanding of their downstream immunological mechanisms to develop new ways of modulating vaccine responses. Here, we review the immunological factors that underlie geographical variation in vaccine responses. Through the identification of causal pathways that link environmental influences to vaccine responsiveness, it might become possible to devise modulatory compounds that can complement vaccines for better outcomes in regions where they are needed most.

Introduction

It is estimated that vaccines have prevented 37 million deaths in the past 20 years[1] , thereby having a substantial impact on global health. However, the full potential of some vaccines is hampered by their low and variable efficacy across populations and geographical areas (Box 1). This was first noted for bacillus Calmette–Guérin (BCG) vaccine efficacy, which was reported to vary with geographical latitude[2]. It is now increasingly recognized that several other vaccines induce variable responses in populations living in different geographical areas or of different socioeconomic status (Fig. 1). These include more recently developed vaccines such as rotavirus vaccines[3,4,5,6] and those under development, such as the whole-organism malaria radiation-attenuated *Plasmodium falciparum* sporozoite (PfSPZ) vaccine[7,8,9,10,11,12] and the PfSPZ–chemoprophylaxis attenuated vaccine (PfSPZ–CVac)[12,13,14], which show remarkable variation in efficacy. Variable vaccine immunogenicity has been observed when comparing low-income and/or middle-income regions with high-income regions of the world not only for the aforementioned vaccines but also for vaccines that target yellow fever virus[15] and Ebola virus[16]. Lower performance of such vaccines, which we refer to as vaccine hyporesponsiveness, is seen not only in low- and middle-income countries, but also in poor rural areas compared with affluent urban regions within the same country[17,18]. It is estimated that worldwide 77 million children receiving BCG and 5 million receiving rotavirus vaccine are insufficiently protected against the diseases targeted by these vaccines[19].

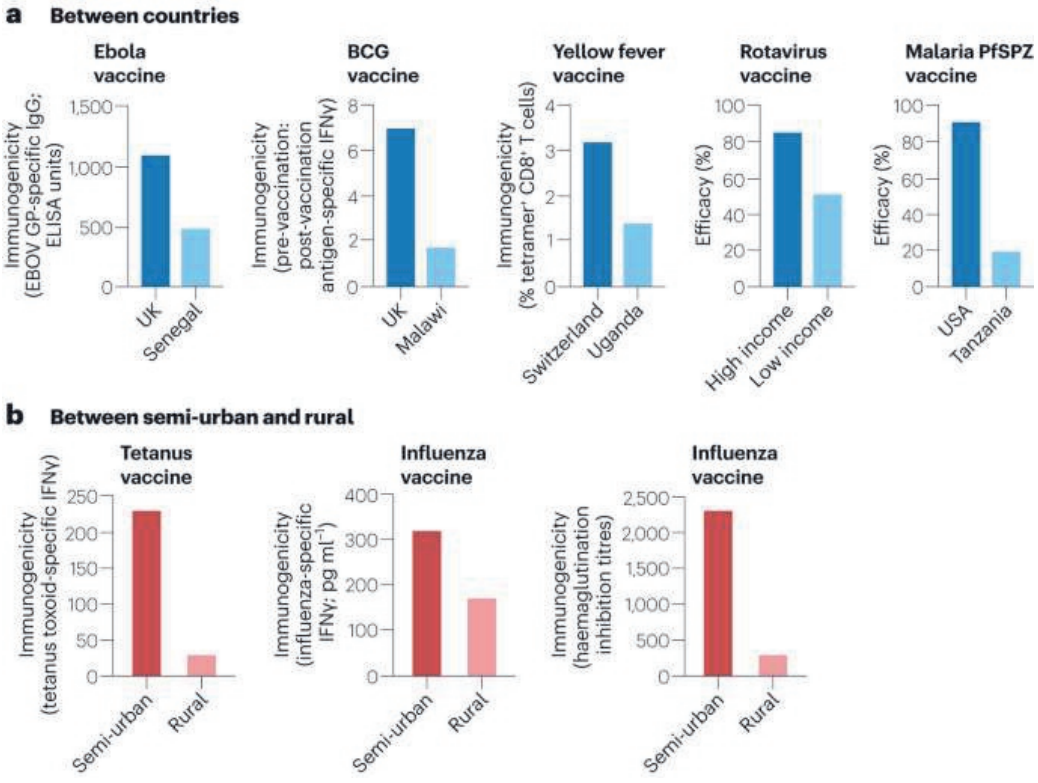

Fig. 1: Variations in vaccine immunogenicity or efficacy across populations.

a, Vaccine immunogenicity varies between countries. The immunogenicity of: Ebola vaccine in the UK and Senegal was assessed by specific IgG antibodies[89]; bacillus Calmette–Guérin (BCG) vaccine in the UK and Malawi was assessed by the increase in interferon-γ (IFNγ) production in response to tuberculin purified protein derivative from pre-vaccination to post-vaccination[148]; yellow fever vaccine in Switzerland and Uganda was determined by the percentage of yellow fever antigen-specific tetramer-positive $CD8^+$ T cells[15]; rotavirus vaccine in high- and low-income countries was assessed by vaccine efficacy6; and irradiated malaria *Plasmodium falciparum* sporozoite (PfSPZ) vaccine in the USA[8] and Tanzania[11] was assessed by vaccine efficacy. **b**, The immunogenicity of vaccines varies between semi-urban and rural settings. In semi-urban and rural Gabon, tetanus vaccine was assessed by tetanus toxoid-stimulated IFNγ production by peripheral blood mononuclear cells (PBMCs)[149]; influenza vaccine was assessed by either influenza virus-stimulated IFNγ production by PBMCs or antibody titres through the haemagglutination inhibition assay[18]. EBOV, Ebola virus; ELISA, enzyme-linked immunosorbent assay; GP, envelope glycoprotein.

Although genetic factors hard wire immune and vaccine responsiveness[20], twin studies have indicated that non-heritable factors contribute by more than 70% to shaping the immune response to vaccines[21,22]. Numerous environmental and non-heritable factors have been implicated in vaccine hyporesponsiveness, including nutritional status, the microbiome and

exposure to microorganisms and parasites[23,24,25,26,27,28] (Box 2). However, interventions to target some of these factors, such as micronutrient supplementation and/or probiotics[29] and anthelmintic treatment[30,31], have had variable success. This highlights that vaccine responses are modulated by multiple factors, which poses a challenge in applying public health measures to overcome vaccine hyporesponsiveness. Therefore, it is important to understand the mechanisms through which environmental factors drive vaccine hyporesponsiveness. Advances in technologies such as transcriptomics, metabolomics and epigenetic analyses at the single-cell level as well as high-dimensional cytometry allow us to study vaccine-specific immune responses in greater breadth and depth (Box 3), helping to identify new pathways and networks of immunological events that can be targeted for more effective vaccines[32,33].

In this Review, we discuss the immunological factors and proposed mechanisms that underlie variation in efficacy or immunogenicity of vaccines across populations from different geographical areas. We largely focus on vaccines against tuberculosis, rotavirus gastroenteritis, yellow fever and malaria, which are worldwide, highly prevalent and life-threatening infectious diseases [Box 1].

Box 1 Vaccine hyporesponsiveness: efficacy and immunogenicity

Vaccine performance can be studied through the assessment of immunogenicity, efficacy or effectiveness. Immunogenicity reveals the extent of an immune response evoked by a vaccine, whereas efficacy and effectiveness assess the beneficial effects of the vaccine in a trial setting or under real-life conditions, respectively. Immunogenicity, in contrast to efficacy and effectiveness, can be studied all over the world irrespective of whether the target disease is prevalent in a particular area and requires only a limited number of vaccinated individuals. However, with the increasing use of safe controlled human infection models in testing vaccines, it is now also possible to assess vaccine efficacy in different populations and geographical areas[11,150,151,152]. In such studies, healthy volunteers are given a vaccine or a placebo and thereafter are challenged with an infectious dose of the target pathogen that is known to establish infection with well-defined time to patency, burden and/or symptoms. Through this approach any protective effect of the administered vaccine against the given challenge can be assessed. Such models are increasingly complementing the traditional phase I or II studies in the field[150], although these cannot fully replace placebo-controlled trials. Differences in immunogenicity and efficacy of both licensed and newly developed vaccines

have become apparent between populations living in geographical areas that differ in environmental and socioeconomic conditions.

Tuberculosis

The bacillus Calmette–Guérin (BCG) vaccine is currently the only tuberculosis vaccine approved and licensed for use, and it consists of live attenuated *Mycobacterium bovis*[153]. This vaccine is recommended to be given at birth in 157 countries[154], and its protective efficacy has largely been attributed to $CD4^+$ T cell-mediated immunity that can stimulate monocytes and/or macrophages to destroy intracellular mycobacteria; however, $CD8^+$ T cell-mediated cytotoxicity towards mycobacteria-infected cells has also been shown[155]. The efficacy of the BCG vaccine progressively increases further from the equator; with 23% efficacy at less than 20 degrees latitude, 32% at 20–40 degrees latitude and 69% at more than 40 degrees latitude[156][Supplementary Table 1].

Rotavirus gastroenteritis

The rotavirus vaccine developed to protect against severe diarrhoea is a live attenuated vaccine that is administered orally[157]. The first vaccine dose is given before 15 weeks of age, followed by one or two additional doses before 8 months of age[158]. It mediates protection mainly through the generation of antibodies to rotavirus[159]. The highest efficacy of Rotarix, one of the currently licensed rotavirus vaccines, over the first year of life, was seen in high-income countries (>95%), whereas this was lower in middle-income countries (>80%) and low-income countries (<75%), with the lowest reported performance in Malawi (49.2%)[26,160]. This trend was confirmed in a meta-analysis[161] (Supplementary Table 2). The recently developed rotavirus RV3-BB vaccine showed a high cumulative serum immune response (76%) in neonates in Java, the most well-developed island of Indonesia[162]; however, in Malawi, the cumulative serum IgA seroconversion rate was 57% for neonates and 59% for infants 4 weeks after vaccination[163], which resembles the seroconversion rate of Rotarix in Malawian infants (57%)[164].

Yellow fever

The yellow fever vaccine is a live attenuated vaccine (17D strain) that can be given from the age of 9 months[165], and it protects by generating neutralizing antibodies[166]. Such live attenuated vaccines replicate and thus mimic a natural infection, which leads to a prolonged activation of multiple innate immune pathways and can induce appropriate humoral and cellular responses[167]. Although there were no vaccine efficacy studies performed, the vaccine is considered highly effective, and immunogenicity determined by seroconversion

rates after vaccination was statistically significantly higher in Europe and the USA (99%) than in Latin American countries, including Brazil and Colombia (94%)[168]. Furthermore, a study that compared 9-month-old children reported that the seroconversion rate was lower in rural Ghana (63.8%) than in urban Mali (91.0%)[44] (Supplementary Table 3). A comparison of immunological responses to yellow fever vaccines in Switzerland and Uganda noted that, although antibody titres reached protective levels in both cohorts, individuals from Switzerland had significantly higher titres of neutralizing antibodies than individuals from Uganda[15].

Malaria

RTS,S is a subunit malaria vaccine adjuvanted with AS01, which is the only licensed malaria vaccine and is given in four doses to children from 5 months of age in areas of moderate and high malaria transmission[169]. It leads to antibody responses to circumsporozoite protein on sporozoites[170]. RTS,S/AS01 showed promising efficacy in malaria-naive adults[171]; however, variable efficacy was reported in a large phase III clinical trial in seven African countries, with an average 36% efficacy after three doses and booster regime in children aged 5–17 months[172] (Supplementary Table 4). Whole-sporozoite vaccines such as the live *Plasmodium falciparum* sporozoite (PfSPZ) vaccine are currently under development. This vaccine is ultimately to be given to children from 6 months of age[173], and it works through the induction of $CD8^+$ T cell responses that target *P. falciparum*-infected hepatocytes146. In controlled human infection, in American malaria-naive subjects of various ethnic backgrounds, PfSPZ vaccine protected 12 of the 13 recipients (92.3%), whereas in a malaria-endemic area in Tanzania it protected only 4 of the 20 recipients (20%)[8,11]. Moreover, efficacy was shown to be much lower in a setting of natural infection in Mali [9,10]. Recent studies of PfSPZ–chemoprophylaxis attenuated vaccine (PfSPZ–CVac), which is a live chemo-attenuated *P. falciparum* vaccine, protected 100% of Dutch[174] and German[13] volunteers, whereas double the dose protected only 8 of the 13 recipients (55%) in Equatorial Guinea[12] [Supplementary Table 5].

Box 2 Linking environmental factors to varied vaccine responses is complex

Variations in vaccine response have been linked to exposure to and/or infections with viruses (for example, cytomegalovirus)[16], environmental mycobacteria[156] and parasites (such as helminths)[31,175]. However, confirming the impact of a single pathogen on vaccine responses is complicated; indeed, treatments that target a single type of pathogen, for example, anthelmintics, have had variable success[30,31]. This might be due to co-infections that are not removed by the given treatment or by incomplete reversal of the effect of past exposure by the treatment1[14,115,176].

High exposure to pathogens is often coincident with other key factors that influence vaccine responsiveness, such as malnutrition or altered gut microbiome composition. For example, helminth infections are often associated with poor nutritional status[177,178], as well as altered microbiome composition[179,180]. Poor nutritional status negatively impacts the immune system[181,182,183], and new insight into the links between the diet, the microbiome and the immune system indicate that even well-nourished individuals may have altered vaccine responses via mechanisms that involve food-derived metabolites that originate from dietary intake, such as flavonoids[133].

The association between microbiome composition and vaccine responses has been studied for several vaccines, including the rotavirus vaccine[24,36,184]. A recent study showed that several bacterial taxa (such as *Streptococcus* and Enterobacteriaceae) positively correlate with rotavirus seroconversion, whereas phage diversity, enterovirus B and multiple cosaviruses were negatively associated[184]. However, in a multicentre cohort study, microbiota diversity was negatively associated with neonatal rotavirus vaccine seroconversion in infants from India but not in infants from the UK, but no specific bacterial taxa could be linked to vaccine outcome in this case[36]. In addition to these associations, in one intervention study, antibiotics were administered before influenza vaccination, which reduced antibody induction in subjects with low pre-existing immunity to influenza virus and who had not been exposed to the influenza vaccine in the preceding 3 years. Antibiotic treatment had little effect if vaccinees had higher pre-vaccination antibodies and therefore showed lower seroconversion rates. This suggests that the microbiome has an adjuvant effect on the antibody response to vaccination in individuals with relatively little prior exposure to the antigen, but that immune memory caused by prior exposure to the antigen can withstand even the most severe perturbation of the microbiome[185]. Larger studies are needed to confirm these findings, and it remains to be determined whether such perturbation would affect other vaccine responses. Moreover, a

causal link showing an effect on vaccine responses by faecal microbiome transplantation[186] or introduction of a combination of microbiota species is lacking.

Taken together, these studies highlight the fact that multiple factors have a role in modulating responses to vaccines and indicate how complex it might be to intervene at the level of environmental factors. Therefore, it is crucial to fully understand the downstream impact of environmental exposures on the immune system to identify immunological traits that are linked to, and could be targeted to improve, vaccine hyporesponsiveness.

Box 3 High-dimensional methods to predict vaccine responses

Differences in response to vaccination may in part be due to variations in baseline or early post-vaccination immune signatures. By combining high-dimensional immunological data with mathematical and computational analyses, it has been possible to define early signatures that predict vaccine immunogenicity, analysis that has mostly been done in cohorts in the USA. Studies of immune responses after vaccination showed that the generalizability of immune signatures was limited; predictive signatures for one particular vaccine could not predict outcomes for other vaccines[187,188]. A meta-analysis study sought to identify universal predictors of vaccine-induced responses with data from 820 adults in 28 studies against 13 different vaccines. They found a consistent association between peak plasmablast levels and antibody induction after vaccination, but there was no other common signature that predicted a response to all vaccines; the responses depended on vaccine type and adjuvant type administered[189].

Similar analyses of baseline samples (before vaccination) have also been carried out to predict the outcome of vaccination[190] (see the table). The first study integrated microRNA and transcriptomic profiling to predict responses to a seasonal influenza vaccine in young adults, older individuals and individuals with diabetes across seasons and showed that immune signatures at baseline could distinguish between high and low vaccine responders[191]. Plasmablast and innate immunity modules at baseline predicted influenza-specific antibody levels at 1 month after vaccination, but not the longevity of the response. Baseline signatures of T and B cell gene modules correlated positively, whereas a monocyte inflammatory signature correlated negatively with antibody responses at 1 month, but showed little correlation with longevity of the response. This landmark study was followed by a combined effort examining six influenza vaccine cohorts that spanned distinct locations, ages and

seasons[192]. Nine genes and three gene modules were found to be associated with the magnitude of the antibody response in all study cohorts. Analysis in independent cohorts validated the baseline signatures predicting responses in young adults, but surprisingly, they had an inverse correlation in older adults[192].

Using a similar systems biology approach, Kotliarov and coworkers[193] identified a signature that predicts both influenza and yellow fever vaccine outcome. Ten genes involved in type I interferon responses were identified in immune cells at baseline that predicted antibody levels in three out of four influenza vaccine trials, as well as the antibody response to the yellow fever vaccine[193]. Another recent study analysed pre-vaccination transcriptome data of 820 adults from different vaccination studies[194]. Taking an unbiased approach, a common pre-vaccination transcriptional signature with an overall predictive value of 62.3% for 13 different vaccines was identified, although the performance varied with different vaccines. The predictor consisted of an inflammatory gene signature downstream of nuclear factor-κB (NF-κB) and interferon regulatory factor 7 in the innate immune cell compartment. Of interest, the inflammatory signature did not predict vaccine responses in elderly individuals, suggesting that the type of inflammation reflected by the signature in this age group has a different origin[194] (see the table). Given that the signalling networks regulated by NF-κB are enhanced in inflammageing[195], these results also suggest that the extent of the activation of these networks might be crucial: their activation favours vaccine responses, yet their overactivation hampers vaccine responses.

Recent pioneering studies of large numbers of children and infants who were protected from clinical malaria following vaccination with RTS,S/AS01E (phase III trial) have shown that signatures that include NF-κB, Toll-like receptors and monocyte-related blood transcriptional modules, in baseline peripheral blood mononuclear cell cultures, depending on type of stimulation, can associate either positively[196] or negatively[197] with vaccination outcomes. Altogether, although systems biology approaches have proved valuable for identifying signatures that predict vaccine outcome, it is not clear how well these signatures hold up across populations from diverse geographical regions with different baseline inflammatory profiles and vaccine responses. Future studies should include a diversity of geographical locations and populations experiencing distinct environmental exposures to determine whether there are shared molecular pathways that underlie vaccine hyporesponsiveness.

Signature at baseline	Predictive for	Study populations (number)	Refs.
Positive correlation: B cell-enriched modules, T cell-enriched modules and T cell surface markers Negative correlation: monocyte-enriched module; cell cycle and its transcriptional regulation	Influenza vaccine Signatures similar across young (<65 years) and older (>65 years) subjects and patients with type 2 diabetes	Discovery cohorts: influenza vaccination from 2007, 2008, 2009, 2010 and 2011 ($n = 212$), including older subjects ($n = 54$) and patients with type 2 diabetes ($n = 17$) Validation cohorts: influenza vaccination from 2008 and 2009 ($n = 218$)	[191]
Positive correlation (in subjects <35 years; negatively correlated in subjects >65 years): B cell receptor signalling, cell structure and motility, inflammatory responses and platelet activation	Influenza vaccine	Discovery cohorts: influenza vaccination from 2008, 2010, 2011 and 2012 ($n = 293$), including young (<35 years) and older (>65 years) adults Validation cohorts: influenza vaccination from 2009 and 2010 ($n = 223$)	[192]
Positive correlation: activated B cells ($CD20^{+}CD38^{++}$), cell cycle activation, type I interferon response Negative correlation: effector memory $CD4^{+}$ T cells	Influenza vaccine, yellow fever vaccine (YF-17D), systemic lupus erythematosus Independent of age	Discovery cohort: influenza vaccination ($n = 63$) Validation cohorts: influenza vaccination from 2008, 2011 and 2012 ($n = 42$); yellow fever vaccination from two trials ($n = 22$) Systemic lupus erythematosus cohort ($n = 34$)	[193,198]
Positive correlation: interferon-stimulated genes and pro-inflammatory genes, such as innate immune sensors in monocytes and dendritic cells Negative correlation: transcriptomic markers of natural killer cells, T	13 vaccines against influenza virus, yellow fever, HIV, Ebola virus, malaria, hepatitis A virus, hepatitis B virus, tuberculosis, smallpox, meningococcus, pneumococcus	Training on the entire cohort ($n = 820$), transcriptional profiles revealed three endotypes: high, middle and low inflammatory; immune subsets and antibody responses were compared	[194]

cells and B cells; target genes of pathways involved in cell proliferation and metabolism (*E2F* and *MYC*)		between these endotypes	
Positive correlation: B cell activation Negative correlation: inflammation, effector memory $CD4^+$ T cells (CD28)	Hepatitis B virus vaccine Signature correlated with age	First approach: entire cohort of adults aged 25–83 years ($n = 174$) Second approach: training cohort ($n = 116$) and test cohort ($n = 58$)	[48]

Immunological factors linked to vaccine hyporesponsiveness

Several immunological contexts may underlie vaccine hyporesponsiveness, including pre-existing immunity, exuberant immune activation, skewed immune responses and restructured lymphoid tissue [Fig. 2], and may explain the varied efficacy of vaccines between different geographical areas and populations.

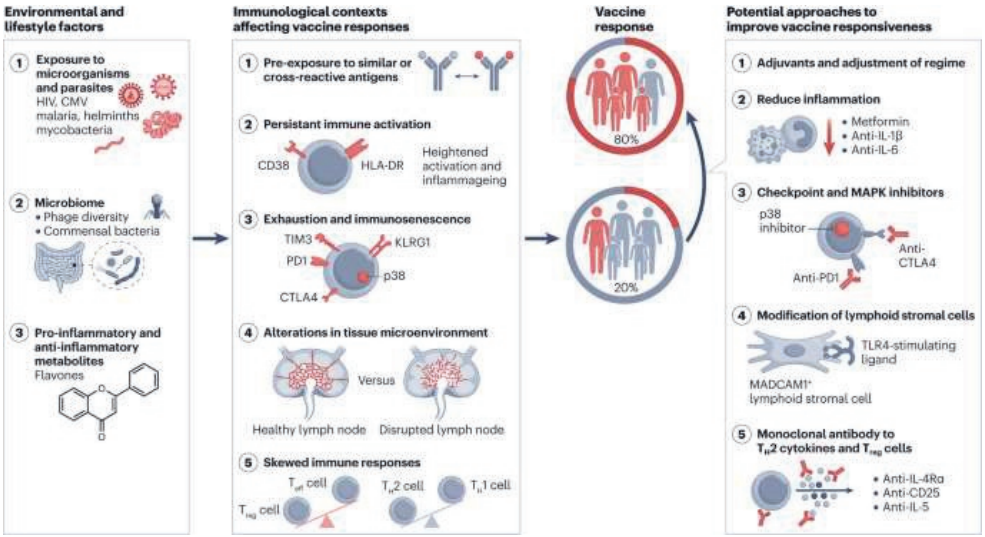

Fig. 2: Factors and immunological mechanisms driving vaccine efficacy variation between populations.

Immune reactivity to vaccines is shaped by previous exposure to several environmental and lifestyle factors. Immunological contexts that negatively affect vaccine responses include pre-existing immunity that results from exposure to similar or cross-reactive antigens, persistent challenges of the immune system that lead to naive T cell depletion, heightened immune activation, immune exhaustion and immunosenescence that impair the response to vaccines, restructuring of the lymphoid tissue and skewing of the immune system. This may be reflected in the different vaccine efficacies observed between different populations. Approaches to overcome vaccine

hyporesponsiveness could be envisaged through various immunological interventions. CMV, cytomegalovirus; KLRG1, killer cell lectin-like receptor subfamily G member 1; MADCAM1, mucosal address in cell adhesion molecule 1; T_{eff} cell, effector T cell; T_H1, T helper 1 cell; T_H2, T helper 2 cell; TLR4, Toll-like receptor 4; T_{reg} cell, regulatory T cell.

Pre-existing immunity to similar or cross-reactive antigens

One of the most intensively discussed effects of pre-exposure to a pathogen on performance of a vaccine that targets the pathogen has been the impact of environmental mycobacteria on BCG vaccine efficacy. The BCG vaccine consists of live attenuated *Mycobacterium bovis*, which mainly infects cattle but is closely related to the human pathogen *Mycobacterium tuberculosis*, and its protective effect can be affected by interference of cellular immune responses to non-tuberculous mycobacteria in the environment[2]. The mechanism that underlies this interference has been hypothesized to be via either a 'blocking' or 'masking' mechanism. According to the blocking hypothesis, pre-existing immune responses accelerate the clearance of BCG by preventing the multiplication of live attenuated bacteria required for the induction of an effective vaccine response. Essential to this hypothesis is that exposure to non-tuberculous mycobacteria induces no or little protection against tuberculosis. The masking hypothesis postulates that exposure to non-tuberculous mycobacteria provides significant protection against tuberculosis and thereby masks the effect of BCG, as vaccine efficacy is calculated by comparing disease incidence between vaccinated and unvaccinated individuals[34]. Although these two hypotheses are not mutually exclusive, a study by Barreto et al.[35] supports the notion that blocking rather than masking is the predominant mechanism behind the geographical variation in BCG vaccine efficacy.

Interestingly, the blocking hypothesis might apply to the rotavirus vaccine, as children with higher titres of maternal anti-rotavirus IgG have a lower seroconversion rate after vaccination[36,37,38]. Similarly, the blocking effect of pre-exposure on vaccine responses seems to have a role in the reduced immunogenicity of the RTS,S malaria subunit vaccine. Analysis of data from a phase III trial of the RTS,S/AS01E vaccine showed that high levels of pre-vaccination antibodies to circumsporozoite epitopes were associated with low levels of vaccine-induced antibodies, particularly in infants[39]. The same principle might apply to the superior immunogenicity of a malaria vaccine candidate, the RH5.1 antigen, when boosting is delayed. The delayed boosting schedule corresponded to a time point when antibody levels from previous doses were declining[40]. Mechanistically, the binding of pre-existing

antibodies to vaccine antigens in the lymph nodes (LNs) could interfere with boosting of vaccine-induced antibody responses[41]. With respect to the efficacy of live attenuated malaria vaccines, which are associated with cellular immune responses that target liver-stage parasites, pre-exposure to malaria parasites might also have a role[28], but in a different way. Through repeated exposure to malaria parasites, both enhanced innate immunity through a type I interferon response and memory liver-resident $CD8^+$ T cells can impede the entry of vaccine-delivered sporozoites to the liver, thereby reducing the induction of protective immune responses[42,43]. However, pre-vaccination antibody titres against yellow fever virus do not seem to have a role in reduced responses to the yellow fever vaccine, as yellow fever vaccination resulted in higher seroconversion in Mali (91.0%) than in Ghana (63.5%) even though the pre-vaccination antibody titres were higher in Mali and, indeed, were not associated with post-vaccination antibody titres[44]. As the yellow fever vaccine induces an extremely robust protective response, and a fractional dose of this vaccine induces strong immunity[45], it is possible that the ability of the vaccine to self-replicate is not sufficiently hampered by the pre-existing neutralizing antibodies. A full understanding of pre-existing immunity could enable a better design in terms of selecting adjuvants, targeting of multiple epitopes or timing of boosting to help overcome any blocking effects on vaccine performance [Fig. 2].

Heightened inflammation and immune activation

The activation status of the immune system before vaccination is of great importance to the quality of the induced immune response. In high-income countries, poor responses to some vaccines in elderly subjects have long been recognized and attributed to dysregulated immune interactions[46,47], raising the question of whether there are immunological commonalities with younger populations of low- and middle-income countries where vaccine hyporesponsiveness is seen. In elderly individuals, age-related alterations, such as lifelong exposure to immunological triggers and reduced ability of immune cell self-renewal, result in smaller naive T and B cell pools, which along with low-grade sterile inflammation, can underlie poor responses to vaccines[48,49]. In many areas of low- and middle-income countries, the continued challenge of the immune system, largely through exposure to microorganisms and parasites starting early in life, can lead to inflammation and a state of heightened activation of both innate and adaptive immune cells[50,51], clonal expansion and depletion of the naive lymphocyte pool[52], impairing the immune response to vaccination. Therefore, persistent inflammation and continuous reactivation of immune cells can result in

immune exhaustion and immunosenescence. These terms, which are often used indiscriminately, represent still not fully understood[53], distinct, yet overlapping, processes that mark an immune state that is detrimental to the outcome of vaccination. Detailed understanding of the characteristics of different states of the immune system associated with vaccine hyporesponsiveness might be helpful for designing interventions to improve vaccine performance.

Gradual loss of naive T and B cells occurs naturally with ageing, but variation in their numbers has also been observed between various aged-matched populations from various geographical locations with different levels of exposure to infections[54,55,56]. For example, a study of age-matched children from Bangladesh and the USA found considerable similarity in immune profiles in the first year of life but at the age of 2–3 years, children from Bangladesh had higher numbers of differentiated $CD4^+$ T cells and fewer monocytes and naive T cells compared with their counterparts from the USA. Importantly, T cell maturity in children from Bangladesh resembled that of adults in the USA[54]. These results are in line with studies showing that Malawian adolescents (aged 12–15 years) had a lower percentage of naive $CD4^+$ and $CD8^+$ T cells ($CD45RO^-CD62L^{hi}CD11a^{low}$) than their UK counterparts. The percentage of naive T cells was negatively associated with cytomegalovirus seropositivity, which was more common in Malawian populations (100%) than in UK populations (36%)[55]. Also comparing immune profiles of individuals living in rural and urban areas of Senegal with those in the Netherlands showed a gradient in the proportion of naive T and B cells in young adults, with the lowest in rural Senegal, then urban Senegal followed by the Netherlands. This correlates with the highest exposure to microorganisms and parasites in rural Senegal and the lowest in the Netherlands[52]. Lower naive T cell numbers before vaccination have been associated with reduced responses to attenuated vaccinia virus in non-human primates[57], and with lower PfSPZ malaria vaccine-induced antibody responses in a study that compared adult vaccinees from Tanzania and the USA[11].

More recently, acute immune activation has been studied by examining responses following controlled malaria infection in healthy volunteers. It was shown that both *Plasmodium vivax* and *P. falciparum* infection can induce widespread immune activation, affecting myeloid cells and strongly activating 25% of T cells, which were marked by high CD38 expression and low BCL-2 expression[58]. The high level of immune activation has been

observed in individuals with lifelong exposure to malaria[50] alongside lower malaria vaccine responses[11]. The impact of immune activation on vaccine responses has also been reported by Muyanja et al.[15], who studied the baseline immune profiles and vaccine responses to yellow fever vaccine in Uganda and Switzerland. The innate immune compartment was more activated in individuals from Uganda compared with individuals from Switzerland, as evidenced by an increased frequency of activated natural killer (NK) cells ($CD16^{+}HLA\text{-}DR^{+}$), recently activated $CD16^{-}$ NK cells (secreting interferon-γ (IFNγ) after restimulation ex vivo) and pro-inflammatory intermediate monocytes ($CD14^{+}CD16^{+}$), with higher expression of PDL1 and HLA-DR. In addition, in the adaptive arm, both the $CD4^{+}$ and $CD8^{+}$ T cell and B cell compartments exhibited more differentiated and memory profiles in individuals in Uganda compared with those in Switzerland. Upon yellow fever vaccination, the frequency of pro-inflammatory monocytes and activated $PD1^{+}CD8^{+}$ T cells at baseline was negatively associated with the induction of neutralizing antibodies, linking the increased immune activation status to impaired vaccination outcome[15].

Needless to say, in children, the length of exposure to environmental factors is shorter and, therefore, the level of immune activation might be less, with little impact on vaccines that are given early in life. However, both rotavirus and cholera vaccines were less effective in children from Bangladesh[59,60]. In a separate study of children from Bangladesh, heightened immune activation was seen at 2 years of age but less so in the first year of life[54], when rotavirus vaccination is given. It would be helpful to assess immunological profiles of children and vaccination outcomes in the same cohorts to conclude with certainty whether immune activation has a role in rotavirus vaccine hyporesponsiveness.

Data generated from immunophenotyping of blood samples from infants and children during the RTS,S malaria vaccine phase III trial was consistent with the idea that the immune system ages at different rates in different geographical areas; however, a more aged or mature immune system in children was associated with a stronger antibody response to RTS,S vaccine[61]. Such discrepancies in how the immune activation status in young children is associated with responses to distinct vaccines highlights the need for more studies: first, to disentangle immune maturation from heightened immune activation; second, to examine local rather than peripheral blood immune profiles, which might be more relevant, for example, for rotavirus vaccine efficacy; and third, to unravel whether different mechanisms underlie hyporesponsiveness to different vaccines. Therefore, a more in-depth understanding of the

mechanisms that underlie, rather than correlates of, vaccine hyporesponsiveness are needed. Given the data generated so far, it would be worth testing strategies to reduce inflammation or heightened immune activation in both elderly individuals and in those living in areas where exposure to microorganisms and parasites is high. This could, for a short period of time, before vaccination, either involve more general drugs, such as metformin, which not only reduces inflammation but also can boost memory formation[62], or more selective compounds that target specific immune pathways such as IL-1β or IL-6 [ref. 63], which have shown some beneficial effects in decreasing inflammation, to potentially reverse vaccine hyporesponsiveness[64] (Fig. 2). However, the benefits and risks associated with such trials will need to be carefully considered given the high infection burden in the environments in which vaccine hyporesponsiveness is often seen to avoid limiting immune control of infections.

Immune exhaustion

Repeated antigenic stimulation of lymphocytes and chronic activation can eventually lead to a state of dysfunction that is broadly termed exhaustion. Exhaustion in various lymphocyte populations, including NK cells, B cells and conventional $CD4^+$ and $CD8^+$ T cells, is generally associated with a progressive hierarchical loss of effector function and proliferative capacity, and the increased expression of inhibitory receptors, such as PD1, CTLA4, LAG3 and TIM3 [refs. 53,65]. However, these inhibitory receptors are also transiently upregulated on functional effector T cells after T cell receptor stimulation. Therefore, recent studies of $CD8^+$ T cells at various differentiation stages that identified TOX and eomesodermin[66,67] as specific transcription factors that regulate exhaustion might help to better define exhausted T cells[53]. Immune exhaustion can be caused by several persistent infections, including malaria and those caused by helminth parasites, *M. tuberculosis*, HIV and hepatitis B and C viruses, as well as by cancer[68,69,70,71,72].

Immune cell exhaustion occurring in the context of chronic hepatitis C virus infection was associated with lower antigen-specific T cell responses and seroconversion following hepatitis B vaccination compared with responses in healthy individuals or in individuals who spontaneously cleared hepatitis C virus infection[73]. Although many studies report the upregulation of inhibitory receptors during hepatitis C virus infection, not many studies have linked this upregulation to poor vaccine responses. Comparing hepatitis C virus-infected subjects after hepatitis B vaccination, TIM3 expression on monocytes[74] and PD1-expressing

$CD4^+$ T cells[73] were increased in subjects that did not respond to the vaccine. Chronic exposure to malaria parasites is also associated with alterations in monocytes that might arise from epigenetic changes in precursor cells that reprogramme them towards a less inflammatory phenotype[75], as well as increased expression of PD1 by T cells, suggesting T cell exhaustion[76]. Antibody-mediated blockade of PD1 in in vitro assays improved hepatitis B virus antigen-specific responses[73,77] and malaria antigen-specific responses[78]. Amplification of antigen-specific T cell responses has been shown in vivo when PD1 antagonists were combined with adenovirus-based or irradiated sporozoite-based malaria vaccines in mouse models[79.80].

The combination of immune checkpoint blockade, such as monoclonal antibodies to PD1, PDL1 or CTLA4, and therapeutic cancer vaccines is being studied extensively, but there are very few studies that combine immune checkpoint blockade with vaccines for infectious diseases[81]. However, vaccination against infectious diseases in patients with cancer treated with immune checkpoint blockade is generating some interesting insights. Recent work has shown that a subset of patients with cancer who are undergoing anti-PD1 antibody therapy and are vaccinated for influenza virus show higher increases in circulating $CD4^+$ T follicular helper cells than patients not receiving anti-PD1 treatment[82]. Increases in plasmablasts and antibody titres indicated the potential of anti-PD1 antibody to enhance vaccine responses in humans in the context of immune exhaustion (Fig. 2). Although these findings highlight the potential of using anti-PD1 and other antibodies to immune checkpoints to overcome reduced vaccine efficacy, much more needs to be done to assess the risk of developing strong collateral autoimmune or autoinflammatory responses. Indeed, patients with cancer on anti-PD1 treatment who showed heightened responses to vaccines also had a higher risk of developing immune-related adverse events[82]. Alternative approaches to overcome immune exhaustion, such as the use of Toll-like receptor (TLR) agonists, have been tested in patients on renal replacement therapy who show hyporesponsiveness to vaccines[83]. Indeed, the use of a hepatitis B vaccine with the TLR9 agonist CpG resulted in higher seroprotective antibody titres in patients with chronic kidney disease[84], indicating the ability to improve responses also in the context of immune exhaustion.

Thus, more work is needed to better understand the exhaustion phenotype of T cells as well as of other cell types such as myeloid cells and the mechanisms that underlie its association with vaccine hyporesponsiveness. Studies of exhaustion in the context of cancer show that there are subtypes of exhausted T cells — TCF1- exhausted T cells and self-renewing $TCF1^+$ stem-like

exhausted T cells[85] — with distinct responses to checkpoint inhibitors, yet very little is known about these subtypes during chronic exposure to microorganisms and parasites in humans. The same applies to the paucity of information on how repeated exposure to pathogen-associated molecular patterns can alter antigen presentation and the control of responses to vaccination. Blocking the receptors and signalling pathways involved in exhaustion of different immune cells might lead to a degree of reversal and enhanced vaccine efficacy, although further studies are needed to assess the safety and benefits of such interventions.

Immunosenescence

Immunosenescence refers to the gradual dysregulation of the immune system as a consequence of ageing, potentially attributed to chronic low-grade antigenic stimulation. It encompasses reduced production of T cells in the thymus, as well as increased sterile, low-grade, chronic inflammation that can contribute to age-associated decline in vaccine efficacy[86]. Although immunosenescence and exhaustion both lead to reduced proliferative capacity and immune function, the pathways involved can be distinct, as reviewed elsewhere[87]. Senescence is characterized by shortening of telomeres, loss of telomerase activity and expression of CD57 and killer cell lectin-like receptor subfamily G member 1 (KLRG1)[87, although CD57 and KLRG1 can also be co-expressed with exhaustion markers such as PD1 [ref. 88].

In addition to biological ageing, immunosenescence has been associated with latent viruses that might reactivate, such as cytomegalovirus. An immunization study of individuals in the UK and Senegal, involving priming with the chimpanzee adenovirus type 3-vectored Ebola Zaire vaccine (ChAd3-EBO-Z) and boosting with the modified vaccinia Ankara Ebola Zaire-vectored (MVA-EBO-Z) vaccine, found a comparable induction of cytokine-producing T cells but a significantly decreased antibody response in individuals in Senegal compared with the UK[89]. Cytomegalovirus carriage, which was higher in Senegalese, was correlated with increased numbers of phenotypically senescent $CD4^+$ and $CD8^+$ T cells ($CD57^+KLRG1^+$), and the frequency of these cells was negatively associated with the vaccine-specific antibody responses[16]. It is important to note that other infections such as malaria can also contribute to the immunosenescent phenotype seen in Senegalese individuals[90]. The disconnection between comparable T cell cytokine responses yet poorer antibody responses in Senegalese compared with UK vaccinees might be related to the ability of senescent cells to produce cytokines, but this remains to be fully understood.

There is great interest in finding ways to reverse immunosenescence[91], with some progress in animal models using senolytics, such as dasatinib and quercetin, which promote the clearance of senescent cells[92]. Moreover, the control of telomere length in immunosenescent cells is an area that is intensely studied at the molecular level, but currently far from clinical application[93]. However, studies using a p38 mitogen-activated protein kinase inhibitor, losmapimod, in elderly subjects has shown promise in enhancing skin immune reactions to varicella zoster virus antigen[94]. In addition, targeting metabolic pathways, for example, using pan mTOR inhibition by AZD8055, has been shown to reverse senescence in skin fibroblasts[95] and, when the same pathway was targeted in elderly subjects before influenza vaccination, it improved vaccine-induced responses[96]. Yet, to what extent such molecular pathways are specific for senescent cells is largely unknown, as they also affect inflammation [Fig.2].

Therefore, a more precise characterization of overlapping and distinct pathways underlying exhaustion, senescence and heightened activation of the immune system in different human populations is needed to help understand the variation in vaccine responsiveness across geographical areas and design immunological interventions. Are we dealing with a vicious circle of inflammation and regulation that we should disrupt using anti-inflammatory interventions simultaneously with checkpoint blockade for better vaccine outcomes?

Skewed immune responses

The proper functioning of the immune system involves a tight balance between pro-inflammatory and anti-inflammatory responses to allow the development of sufficiently strong immune responses to pathogens yet prevent overzealous inflammation and tissue damage[97,98]. In a simplified view, the immune system deals with a range of pathogens through the induction of various T helper cell subsets, such as T_H1, T_H2 and T_H17 cells, alongside matched innate effector cells, that are suited for optimal control of a particular type of pathogen. These responses are kept in check by regulatory populations, such as regulatory T (T_{reg}) cells, regulatory B cells[99] and anti-inflammatory monocytes or macrophages[100]. T cell responses can also be regulated cell-intrinsically through the upregulation of inhibitory receptors or other molecules that limit their inflammatory activity after activation. For example, in the setting of chronic helminth infection, the protective T_H2 cell responses are compromised by a regulatory environment, generating a so-called 'modified T_H2 cell response' associated with high IL-10 and IgG4 levels and low IgE levels, rather than the typical T_H2 cell

response characterized by high IL-4, IL-5 and IgE[101]. T_{reg} cells can be induced in response to inflammatory signals such as tumour necrosis factor (TNF)[102] but also by certain pathogens that express immunomodulatory molecules to allow their long-term survival within the host[98]. Parasitic helminth infections are highly prevalent in rural areas of low- and middle-income countries and have been shown to be associated with increased numbers of T_H2 cells, group 2 innate lymphoid cells (ILC2s), T_{reg} cells and regulatory B cells[51], which can modulate responses to *P. falciparum* and *M. tuberculosis*[103,104]. A study of T_{reg} cells in an area endemic for helminth infections showed that the suppressive activity of $CD25^{hi}FOXP3^{+}$ T_{reg} cells was higher in helminth-infected children than uninfected children. In vitro T cell proliferative and IFNγ responses to BCG and malaria antigens increased following depletion of T_{reg} cells only in samples from individuals infected with helminths and not in those from uninfected subjects[105]. A role for helminth-induced immune regulation was further substantiated by an anthelmintic trial showing that T cells expressing the inhibitory molecule CTLA4 decreased significantly following the reduction in helminth load, allowing the induction of stronger inflammatory TNF responses to malaria antigens[68]. Similarly, during blood-stage malaria infection, strong regulatory responses have been observed[106,107,108]; the number of T_{reg} cells in the blood positively correlated with blood-stage parasite burden and hampered the development of natural or vaccine-induced protection, as shown in a study that assessed the efficacy of the malaria vaccine candidate GMZ2 using controlled human malaria infection. Moreover, they showed that in addition to increased numbers of T_{reg} cells, levels of HLA-G, which interacts with inhibitory receptors on T cells, B cells, NK cells and neutrophils, were negatively correlated with vaccine-specific antibody concentrations[108].

Given that a T_H1-type and inflammatory status supports vaccine-induced IgG antibody responses, vaccination in the context of a T_H2-type and regulatory environment would be expected to limit vaccine efficacy. A meta-analysis by Wait et al.[109] revealed poorer vaccination outcomes in populations infected by helminths at the time of vaccination. Moreover, the study found that chronic parasite infections, but not acute parasite infections, were associated with worse immunization outcomes[109]. Indeed, a study that examined the immune response to RTS,S vaccination showed that individuals with a T_H1-type and pro-inflammatory response to vaccination (such as production of IFNγ, IL-15 and GM-CSF) were protected from subsequent malaria infection, whereas those that produced the T_H2 cytokine IL-5 were not110. Similarly, a negative association has been found between helminth

infections and protection induced by another malaria vaccine candidate, GMZ2 [ref. 111]. However, anthelmintic treatment has had variable effects on vaccine responses[18,30,31,112,113]. One potential explanation is that the anti-inflammatory immune status is not directly reverted upon helminth removal but can persist[114,115,116].

Altogether, larger studies are needed to delineate the relative contribution of T_H2 and regulatory cells to vaccine hyporesponsiveness and to devise appropriate interventions. The blocking of T_H2 cytokines and their downstream effects has shown promise in the field of asthma, where clinical trials using anti-IL-5 or anti-IL-4Rα show fewer acute exacerbations and reduced eosinophilia[117]. In the field of cancer, there has been significant interest in evaluating the clinical benefits of targeting T_{reg} cells to improve T_H1-type antitumour immune responses. Although success from early clinical trials using the human CD25-specific antibody daclizumab to deplete T_{reg} cells has been modest, more recent approaches using modified antibodies with superior capacity to induce antibody-dependent cell cytotoxicity are more promising[118] [Fig. 2].

Alterations in the lymphoid tissue microenvironment

The lymphoid tissues are essential for correct functioning of the immune system by providing organized structures that support interaction between cells and immune mediators. Structural changes in the LNs have been observed in older individuals, patients with HIV infection (including those on antiretroviral therapy) and healthy individuals from low-income countries (such as Uganda)[119,120,121]. With normal ageing, the number of LNs decreases, and there may be reductions in the area and volume of LN paracortical, cortex and medullary regions[122,123]. Furthermore, naive $CD8^+$ T cell and $CD20^+$ B cell numbers in LNs are reduced and there is a decrease in the relative and absolute dimensions of germinal centres, indicating a more static microarchitecture in older compared with younger individuals[124]. In the context of active HIV-1 replication, inflammation and tissue remodelling cause damage to the LN architecture, limiting its ability to support normal T cell numbers and thereby contributing to the reduced $CD4^+$ T cell numbers observed in these patients[121]. Of interest, examination of LN sections of HIV-negative individuals from Uganda also showed LN architecture disruption, characterized by collagen formation in the parafollicular T cell zone, similar to that observed in HIV-positive individuals from the USA, suggesting that LN remodelling is not limited to HIV infection and may occur with other chronic endemic infections. In addition, the fibroblastic reticular cell network, an essential network for T cell–

antigen interaction, was diminished in HIV-negative Ugandans compared with HIV-negative North Americans, as measured by desmin positivity. Moreover, the depleted fibroblastic reticular cell network was associated with a smaller $CD4^+$ T cell population in the LNs. Vaccination of these HIV-negative Ugandans with the yellow fever vaccine YF-17D resulted in a blunted and short duration antibody response, and the more damage to the fibroblastic reticular cell network the smaller the peak antibody titre. Finally, confocal imaging revealed a lack of T follicular helper cells and diminished B cell follicle formation in HIV-negative Ugandans that was not rescued by vaccination[120].

The importance of an altered lymphoid tissue microenvironment to the development of immune and vaccine responses is also supported by a study that shows that changes to the LN microenvironment during ageing, rather than to the immune cells themselves, contribute to age-related immune dysfunction[124]. In aged mice, lymphoid tissue stromal cells expressing mucosal addressin cell adhesion molecule 1 (MADCAM1) failed to respond to immunization and support germinal centre responses. Targeting TLR4 by adjuvants improved the response to vaccination by $MADCAM1^+$ stromal cells, which correlated with improved germinal centre responses[125] [Fig. 2]. Although alterations in the local microenvironment are receiving more attention lately, more in-depth studies are needed, also in humans, to reverse detrimental alterations in the microenvironment, which appears to be crucial for the vaccine response.

Emerging areas for future of vaccinology

A detailed understanding of the immune system is essential for the development of effective vaccines. However, much of our knowledge of immunology is based on studies carried out in laboratory animals and in humans living in affluent countries, such as the USA or Europe. As the environment has a tremendous impact on the immune system, the future of vaccinology will foremost need to include populations that are exposed to different environments.

Parallels between the immunological changes during cancer and (chronic) infectious diseases might open new possibilities to overcome vaccine hyporesponsiveness. Both advanced cancers and chronic infections can induce persistent activation and inflammation, which can lead to T cell exhaustion, increased numbers of immunosuppressive and regulatory cell populations, as well as a shift from protective T_H1-type immunity to T_H2-type immunity or from pro-inflammatory to anti-inflammatory innate effectors[72,126,127,128]. These changes can compromise the T cell functions necessary for adequate responses to pathogens and tumour

cells as well as to vaccines. Biologics that have been developed for cancer treatment are increasingly being studied in the context of chronic infectious diseases and may be worth exploring to increase vaccine efficacy in those with persistent pathogen exposure[79,80,129,130]. The potential role of the microbiome in enhancing vaccine responses is an emerging area of research, which has been the subject of a recent review[24]. Indeed, a growing number of metabolites derived from the microbiota[131] and foods[132] have been shown to modulate the immune system. A recent study that compared immune responses of residents of urban and rural Tanzania found more anti-inflammatory immune profiles in rural participants, which were associated with increased plasma levels of food-derived flavones[133]. Specifically, the plant-derived flavonoid apigenin showed anti-inflammatory effects reflected in cytokine profiles assessed after cell stimulation[133]. Another study linked iron bioavailability to a reduced response to malaria vaccine (RTS,S) in African children. African children with anaemia had fewer isotype-switched memory B cells and plasmablasts than healthy children, and increasing iron bioavailability in vitro was able to restore the defective B cell proliferation and plasmablast differentiation[61]. With the development of highly sensitive metabolomic and proteomic platforms that better enable specific molecules in biofluids to be linked to immune responsiveness and investigation of the mechanisms that underlie their immunomodulatory effects, it is likely that additional pathways will be discovered as targets for improving vaccine responses.

Previous exposures to microorganisms and parasites are also known to have lasting effects on the innate immune compartment — through processes termed trained immunity and tolerance[134]. Trained immunity refers to a baseline quiescent innate immune cell status that is modulated, at the epigenetic level, by previous exposures, to induce a faster and stronger response to a secondary exposure. Tolerance is the opposite phenomenon by which the response to a secondary exposure is lower than the first. Such a framework (elegantly reviewed recently[134] needs to be dissected precisely to examine whether and/or how it underpins heightened immune activation, exhaustion and senescence and their relation to vaccine hyporesponsiveness.

Another important area of research that can shed light on the mechanisms that govern vaccine hyporesponsiveness and thereby help to identify actionable targets to overcome hyporesponsiveness is the field of immunometabolism. During the past decade it has become increasingly clear that a wide range of immune cell properties, including those leading to trained immunity and tolerance[135], exhaustion[136], senescence[137] and

hyperactivation[138], are associated with and dependent on engagement of particular metabolic programmes. Recent systems vaccinology work linked changes in metabolic pathways to shingles vaccine-induced T and B cell responses[139], and follow-up work in mice pinpointed the importance of sterol metabolism in B cells for antibody production following immunization[140]. These insights have sparked interest in exploring whether immune cell metabolism could be harnessed to direct immune responses for therapeutic gain. These developments are most advanced in the field of cancer, in which targeted modulation of metabolism of tumour-associated myeloid cells and adaptive immune cells has shown promise as a viable means to negate immune dysfunction commonly observed in tumour microenvironments[141,142]. Some of the metabolic principles that underpin immune dysfunction in a tumour context are likely to overlap with those that lead to vaccine hyporesponsiveness, and as such can inform the rational design of approaches that target immune cell metabolism to restore vaccine responsiveness. Efforts in this direction are still in their infancy. However, the clinical trial in which the mTOR inhibitor RAD001 was shown to ameliorate immunosenescence in elderly individuals and improve their response to influenza vaccination[143] provides the first evidence of therapeutic potential of modulation of immune cell metabolism in the context of vaccines. To further this field, a key first step will be to map in detail the metabolic characteristics of immune cell subsets in populations that are affected by poor vaccine responses, to identify therapeutic targets.

Finally, studying compartments other than the peripheral blood seems to be the next frontier in vaccinology. A recent study by Wagar et al.[144] showed how cultures of tonsil tissue can provide a secondary lymphoid organ model to study adaptive immune responses to vaccines. In addition, by taking serial fine needle aspirates of a single LN germinal centre in response to a vaccine over time has provided unique insight into responses to mRNA-based vaccines[145]. Moreover, tissue-resident immune cells studied in malaria vaccine responses of non-human primates highlight that vaccine-induced $CD8^+$ T cells or γδ T cells are present in much higher numbers in the liver, where infected hepatocytes are targeted, than can be appreciated from examining the peripheral blood[146,147]. Studies beyond the peripheral blood also provide the opportunity to examine the stromal cell compartment, which provides essential signals for immune function locally and might also be influenced by, for example, inflammation.

Conclusion

Large-scale omics approaches that combine the study of transcriptomes and proteomes, such as through CITE-seq, show promise for determining baseline vaccine response predictors [Box 3], with further insight now being gained from also assessing epigenomes[33] and metabolomes[139]. Such approaches should also now be applied to cohorts from populations that reside in different environmental settings where exposure to microorganisms and parasites, nutrient and food intake, as well as lifestyle, differ greatly as do vaccine responses. We are hopeful that the dissection of immunological mechanisms that link environment to vaccine responsiveness will unravel pathways that are amenable to modification and identify immunomodulatory compounds that complement vaccines to provide effective vaccination programmes for those who need it most.

References

1. Li, X., Mukandavire, C. & Cucunuba, Z. M. Estimating the health impact of vaccination against ten pathogens in 98 low-income and middle-income countries from 2000 to 2030: a modelling study. Lancet 397, 398–408 (2021).
2. Fine, P. E. M. Variation in protection by BCG-implications of and for heterologous immunity. Lancet 346, 1339–1345 (1995). This publication is the first to address the impact of environmental factors on variation in BCG vaccine efficacy.
3. Vesikari, T. et al. Efficacy of human rotavirus vaccine against rotavirus gastroenteritis during the first 2 years of life in European infants: randomised, double-blind controlled study. Lancet 370, 1757–1763 (2007).
4. Madhi, S. A. et al. Effect of human rotavirus vaccine on severe diarrhea in African infants. N. Engl. J. Med. 362, 289–298 (2010).
5. Clark, A. et al. Efficacy of live oral rotavirus vaccines by duration of follow-up: a meta-regression of randomised controlled trials. Lancet Infect. Dis. 19, 717–727 (2019). This publication is a meta-analysis of trials across the world showing that rotavirus vaccine efficacy and durability are lowest in countries with highest child mortality.
6. Jiang, V., Jiang, B., Tate, J., Parashar, U. D. & Patel, M. M. Performance of rotavirus vaccines in developed and developing countries. Hum. Vaccin. 6, 532–542 (2010).
7. Seder, R. A. et al. Protection against malaria by intravenous immunization with a nonreplicating sporozoite vaccine. Science 341, 1359–1365 (2013).
8. Epstein, J. E. et al. Protection against Plasmodium falciparum malaria by PfSPZ vaccine. J. Clin. Invest. Insight 2, e89154 (2017).
9. Sissoko, M. S. et al. Safety and efficacy of PfSPZ vaccine against Plasmodium falciparum via direct venous inoculation in healthy malaria-exposed adults in Mali: a randomised, double-blind phase 1 trial. Lancet Infect. Dis. 17, 498–509 (2017).
10. Sissoko, M. S. et al. Safety and efficacy of a three-dose regimen of Plasmodium falciparum sporozoite vaccine in adults during an intense malaria transmission season in Mali: a randomised, controlled phase 1 trial. Lancet Infect. Dis. 22, 377–389 (2022).
11. Jongo, S. A. et al. Safety, immunogenicity, and protective efficacy against controlled human malaria infection of Plasmodium falciparum sporozoite vaccine in Tanzanian adults. Am. J. Trop. Med. Hyg. 99, 338–349 (2018). This publication shows that the PfSPZ vaccine when tested in Tanzania results in low (20%) protection against controlled human malaria infection, whereas when tested in the USA, in an identical manner, it is highly protective (92.3%).
12. Jongo, S. A. et al. Immunogenicity and protective efficacy of radiation-attenuated and chemo-attenuated PfSPZ vaccines in EquatoGuinean adults. Am. J. Trop. Med. Hyg. 104, 283–293 (2021).
13. Mordmuller, B. et al. Sterile protection against human malaria by chemoattenuated PfSPZ vaccine. Nature 542, 445–449 (2017).
14. Coulibaly, D. et al. PfSPZ-CVac malaria vaccine demonstrates safety among malaria-experienced adults: a randomized, controlled phase 1 trial. EClinicalMedicine 52, 101579 (2022).
15. Muyanja, E. et al. Immune activation alters cellular and humoral responses to yellow fever 17D vaccine. J. Clin. Invest. 124, 3147–3158 (2014). This publication shows that the immunogenicity of the yellow fever vaccine

is lower in Uganda than in Switzerland and links this to the higher pre-vaccination immune activation status in Uganda.

16. Bowyer, G. et al. Reduced Ebola vaccine responses in CMV+ young adults is associated with expansion of CD57+KLRG1+ T cells. J. Exp. Med. 217, e20200004 (2020). This publication shows that immunogenicity of the Ebola vaccine is lower in Senegal than in the UK and links it to the expression of CD57 and KLRG1 on T cells.
17. Kabagenyi, J. et al. Urban–rural differences in immune responses to mycobacterial and tetanus vaccine antigens in a tropical setting: a role for helminths? Parasitol. Int. 78, 102132 (2020).
18. van Riet, E. et al. Cellular and humoral responses to influenza in gabonese children living in rural and semi-urban areas. J. Infect. Dis. 196, 1671–1678 (2007).
19. Grassly, N. C., Kang, G. & Kampmann, B. Biological challenges to effective vaccines in the developing world. Phil. Trans. R. Soc. B 370, 20140138 (2015).
20. Mentzer, A. J., O'Connor, D., Pollard, A. J. & Hill, A. V. Searching for the human genetic factors standing in the way of universally effective vaccines. Phil. Trans. R. Soc. B 370, 20140341 (2015).
21. Liston, A., Humblet-Baron, S., Duffy, D. & Goris, A. Human immune diversity: from evolution to modernity. Nat. Immunol. 22, 1479–1489 (2021).
22. Brodin, P. et al. Variation in the human immune system is largely driven by non-heritable influences. Cell 160, 37–47 (2015). This publication shows that the variation in immune response of twins to vaccination or an infection is largely determined by exposure to environmental factors.
23. Drakesmith, H. et al. Vaccine efficacy and iron deficiency: an intertwined pair? Lancet Haematol. 8, e666–e669 (2021).
24. Lynn, D. J., Benson, S. C., Lynn, M. A. & Pulendran, B. Modulation of immune responses to vaccination by the microbiota: implications and potential mechanisms. Nat. Rev. Immunol. 22, 33–46 (2022).
25. Shah, J. A., Lindestam Arlehamn, C. S., Horne, D. J., Sette, A. & Hawn, T. R. Nontuberculous mycobacteria and heterologous immunity to tuberculosis. J. Infect. Dis. 220, 1091–1098 (2019).
26. Clarke, E. & Desselberger, U. Correlates of protection against human rotavirus disease and the factors influencing protection in low-income settings. Mucosal Immunol. 8, 1–17 (2015).
27. Parker, E. P. et al. Causes of impaired oral vaccine efficacy in developing countries. Future Microbiol. 13, 97–118 (2018).
28. Morter, R. et al. Impact of exposure to malaria and nutritional status on responses to the experimental malaria vaccine ChAd63 MVA ME-TRAP in 5-17 month-old children in Burkina Faso. Front. Immunol. 13, 1058227 (2022).
29. Lazarus, R. P. et al. The effect of probiotics and zinc supplementation on the immune response to oral rotavirus vaccine: a randomized, factorial design, placebo-controlled study among Indian infants. Vaccine 36, 273–279 (2018).
30. Bruckner, S. et al. Effect of antihelminthic treatment on vaccine immunogenicity to a seasonal influenza vaccine in primary school children in gabon: a randomized placebo-controlled trial. PLoS Negl. Trop. Dis. 9, e0003768 (2015).
31. Tweyongyere, R. et al. Effect of Schistosoma mansoni infection and its treatment on antibody responses to measles catch-up

immunisation in pre-school children: a randomised trial. PLoS Negl. Trop. Dis. 13, e0007157 (2019).

32. O'Connor, D. The omics strategy: the use of systems vaccinology to characterize immune responses to childhood immunization. Expert Rev. Vaccines 21, 1205–1214 (2022).
33. Wimmers, F. et al. The single-cell epigenomic and transcriptional landscape of immunity to influenza vaccination. Cell 184, 3915–3935 (2021).
34. Andersen, P. & Doherty, T. M. The success and failure of BCG — implications for a novel tuberculosis vaccine. Nat. Rev. Microbiol. 3, 656–662 (2005).
35. Barreto, M. L. et al. Causes of variation in BCG vaccine efficacy: examining evidence from the BCG REVAC cluster randomized trial to explore the masking and the blocking hypotheses. Vaccine 32, 3759–3764 (2014). This publication shows data that support the hypothesis that blocking is the most likely explanation for the way in which exposure to environmental mycobacteria decreases BCG vaccine efficacy.
36. Parker, E. P. K. et al. Impact of maternal antibodies and microbiota development on the immunogenicity of oral rotavirus vaccine in African, Indian, and European infants. Nat. Commun. 12, 7288 (2021).
37. Chilengi, R. et al. Association of maternal immunity with rotavirus vaccine immunogenicity in Zambian infants. PLoS ONE 11, e0150100 (2016).
38. Moon, S. S. et al. Prevaccination rotavirus serum IgG and IgA are associated with lower immunogenicity of live, oral human rotavirus vaccine in South African infants. Clin. Infect. Dis. 62, 157–165 (2016)
39. Dobano, C. et al. Concentration and avidity of antibodies to different circumsporozoite epitopes correlate with RTS,S/ASO1E malaria vaccine efficacy. Nat. Commun. 10, 2174 (2019).
40. Nielsen, C. M. et al. Delayed boosting improves human antigen-specific Ig and B cell responses to the RH5.1/AS01B malaria vaccine. JCI Insight 8, e163856 (2023).
41. McNamara, H. A. et al. Antibody feedback limits the expansion of B cell responses to malaria vaccination but drives diversification of the humoral response. Cell Host Microbe 28, 572–585.e7 (2020).
42. Liehl, P. et al. Innate immunity induced by Plasmodium liver infection inhibits malaria reinfections. Infect. Immun. 83, 1172–1180 (2015).
43. Liehl, P. et al. Host-cell sensors for Plasmodium activate innate immunity against liver-stage infection. Nat. Med. 20, 47–53 (2014).
44. Idoko, O. T. et al. Antibody responses to yellow fever vaccine in 9 to 11-month-old Malian and Ghanaian children. Expert Rev. Vaccines 18, 867–875 (2019).
45. Roukens, A. H. & Visser, L. G. Yellow fever vaccine: past, present and future. Expert Opin. Biol. Ther. 8, 1787–1795 (2008).
46. Roukens, A. H. et al. Elderly subjects have a delayed antibody response and prolonged viraemia following yellow fever vaccination: a prospective controlled cohort study. PLoS ONE 6, e27753 (2011).
47. Goodwin, K., Viboud, C. & Simonsen, L. Antibody response to influenza vaccination in the elderly: a quantitative review. Vaccine 24, 1159–1169 (2006).
48. Fourati, S. et al. Pre-vaccination inflammation and B-cell signalling predict age-related hyporesponse to hepatitis B vaccination. Nat. Commun. 7, 10369 (2016).

49. Fali, T. et al. Elderly human hematopoietic progenitor cells express cellular senescence markers and are more susceptible to pyroptosis. JCI Insight 3, e95319 (2018).
50. de Jong, S. E. et al. Systems analysis and controlled malaria infection in Europeans and Africans elucidate naturally acquired immunity. Nat. Immunol. 22, 654–665 (2021).
51. de Ruiter, K. et al. Helminth infections drive heterogeneity in human type 2 and regulatory cells. Sci. Transl Med. 12, eaaw3703 (2020). This publication shows that the immune profiles of Dutch and Indonesian individuals living in Jakarta are more similar than the immune profiles of Indonesians living in Jakarta and a rural village.
52. Mbow, M. et al. Changes in immunological profile as a function of urbanization and lifestyle. Immunology 143, 569–577 (2014).
53. Blank, C. U. et al. Defining 'T cell exhaustion'. Nat. Rev. Immunol. 19, 665–674 (2019).
54. Wagar, L. E. et al. Increased T cell differentiation and cytolytic function in Bangladeshi compared to American children. Front. Immunol. 10, 2239 (2019).
55. Ben-Smith, A. et al. Differences between naive and memory T cell phenotype in Malawian and UK adolescents: a role for cytomegalovirus? BMC Infect. Dis. 8, 139 (2008).
56. Messele, T. et al. Reduced naive and increased activated CD4 and CD8 cells in healthy adult Ethiopians compared with their Dutch counterparts. Clin. Exp. Immunol. 115, 443–450 (1999).
57. Cicin-Sain, L. et al. Loss of naive T cells and repertoire constriction predict poor response to vaccination in old primates. J. Immunol. 184, 6739–6745 (2010).
58. Bach, F. A. et al. A systematic analysis of the human immune response to Plasmodium vivax. J. Clin. Invest. 133, e152463 (2023).
59. Rogawski, E. T. et al. quantifying the impact of natural immunity on rotavirus vaccine efficacy estimates: a clinical trial in Dhaka, Bangladesh (PROVIDE) and a simulation study. J. Infect. Dis. 217, 861–868 (2018).
60. Qadri, F. et al. Efficacy of a single-dose, inactivated oral cholera vaccine in Bangladesh. N. Engl. J. Med. 374, 1723–1732 (2016).
61. Hill, D. L. et al. Immune system development varies according to age, location, and anemia in African children. Sci. Transl Med. 12, eaaw9522 (2020). This publication shows how that the trajectory of immune activation can vary considerably in infants between different geographical locations and this can impact vaccine responses.
62. Frasca, D., Diaz, A., Romero, M. & Blomberg, B. B. Metformin enhances B cell function and antibody responses of elderly individuals with type-2 diabetes mellitus. Front. Aging 2, 715981 (2021).
63. Ridker, P. M. et al. Effects of interleukin-1beta inhibition with canakinumab on hemoglobin A1c, lipids, C-reactive protein, interleukin-6, and fibrinogen: a phase IIb randomized, placebo-controlled trial. Circulation 126, 2739–2748 (2012).
64. Ferrucci, L. & Fabbri, E. Inflammageing: chronic inflammation in ageing, cardiovascular disease, and frailty. Nat. Rev. Cardiol. 15, 505–522 (2018).
65. McLane, L. M., Abdel-Hakeem, M. S. & Wherry, E. J. CD8 T cell exhaustion during chronic viral infection and cancer. Annu. Rev. Immunol. 37, 457–495 (2019).
66. Wildner, N. H. et al. Transcriptional pattern analysis of virus-specific CD8+ T cells in hepatitis C infection: increased expression of

TOX and eomesodermin during and after persistent antigen recognition. Front. Immunol. 13, 886646 (2022).

67. Beltra, J. C. et al. Developmental relationships of four exhausted CD8+ T cell subsets reveals underlying transcriptional and epigenetic landscape control mechanisms. Immunity 52, 825–841.e8 (2020). By using transcriptional and epigenetic analysis, this publication identifies dedicated molecular signatures that define the heterogeneity of exhausted CD8+ T cells.
68. Wammes, L. J. et al. Community deworming alleviates geohelminth-induced immune hyporesponsiveness. Proc. Natl Acad. Sci. USA 113, 12526–12531 (2016).
69. Labuda, L. A. et al. A praziquantel treatment study of immune and transcriptome profiles in Schistosoma haematobium-infected Gabonese schoolchildren. J. Infect. Dis. 222, 2103–2113 (2020).
70. Jayaraman, P. et al. TIM3 mediates T cell exhaustion during Mycobacterium tuberculosis infection. PLoS Pathog. 12, e1005490 (2016).
71. Barili, V. et al. Unraveling the multifaceted nature of CD8 T cell exhaustion provides the molecular basis for therapeutic T cell reconstitution in chronic hepatitis B and C. Cells 10, 2563 (2021).
72. Hotchkiss, R. S. & Moldawer, L. L. Parallels between cancer and infectious disease. N. Engl. J. Med. 371, 380–383 (2014).
73. Moorman, J. P. et al. Impaired hepatitis B vaccine responses during chronic hepatitis C infection: involvement of the PD-1 pathway in regulating CD4+ T cell responses. Vaccine 29, 3169–3176 (2011).
74. Wang, J. M. et al. Tim-3 alters the balance of IL-12/IL-23 and drives TH17 cells: role in hepatitis B vaccine failure during hepatitis C infection. Vaccine 31, 2238–2245 (2013).
75. Guha, R. et al. Plasmodium falciparum malaria drives epigenetic reprogramming of human monocytes toward a regulatory phenotype. PLoS Pathog. 17, e1009430 (2021).
76. Illingworth, J. et al. Chronic exposure to Plasmodium falciparum is associated with phenotypic evidence of B and T cell exhaustion. J. Immunol. 190, 1038–1047 (2013).
77. Bengsch, B., Martin, B. & Thimme, R. Restoration of HBV-specific CD8+ T cell function by PD-1 blockade in inactive carrier patients is linked to T cell differentiation. J. Hepatol. 61, 1212–1219 (2014).
78. Edwards, C. L. et al. Early changes in CD4+ T-cell activation during blood-stage Plasmodium falciparum infection. J. Infect. Dis. 218, 1119–1129 (2018).
79. Kotraiah, V. et al. Novel peptide-based PD1 immunomodulators demonstrate efficacy in infectious disease vaccines and therapeutics. Front. Immunol. 11, 264 (2020).
80. Phares, T. W. et al. A peptide-based PD1 antagonist enhances T-cell priming and efficacy of a prophylactic malaria vaccine and promotes survival in a lethal malaria model. Front. Immunol. 11, 1377 (2020).
81. Batista-Duharte, A., Hassouneh, F., Alvarez-Heredia, P., Pera, A. & Solana, R. Immune checkpoint inhibitors for vaccine improvements: current status and new approaches. Pharmaceutics 14, 1721 (2022).
82. Herati, R. S. et al. PD-1 directed immunotherapy alters Tfh and humoral immune responses to seasonal influenza vaccine. Nat. Immunol. 23, 1183–1192 (2022). This publication shows that anti-PD1 therapy might be useful for boosting non-

cancer immune responses as it resulted in improved T folicular helper cell responses following influenza vaccination.

83. Lindemann, M. et al. Humoral and cellular responses to a single dose of fendrix in renal transplant recipients with non-response to previous hepatitis B vaccination. Scand. J. Immunol. 85, 51–57 (2017).
84. Janssen, R. S. et al. Immunogenicity and safety of an investigational hepatitis B vaccine with a toll-like receptor 9 agonist adjuvant (HBsAg-1018) compared with a licensed hepatitis B vaccine in patients with chronic kidney disease. Vaccine 31, 5306–5313 (2013).
85. Siddiqui, I. et al. Intratumoral Tcf1+PD-1+CD8+ T cells with stem-like properties promote tumor control in response to vaccination and checkpoint blockade immunotherapy. Immunity 50, 195–211 (2019).
86. Chen, J. D. Y., Deng, J. C. & Goldstein, D. R. How aging impacts vaccine efficacy: known molecular and cellular mechanisms and future directions. Trends Mol. Med. 28, 1100–1111 (2022).
87. Akbar, A. N. & Henson, S. M. Are senescence and exhaustion intertwined or unrelated processes that compromise immunity? Nat. Rev. Immunol. 11, 289–295 (2011)
88. Bengsch, B. et al. Coexpression of PD-1, 2B4, CD160 and KLRG1 on exhausted HCV-specific CD8+ T cells is linked to antigen recognition and T cell differentiation. PLoS Pathog. 6, e1000947 (2010).
89. Venkatraman, N. et al. Safety and immunogenicity of a heterologous prime-boost Ebola virus vaccine regimen in healthy adults in the United Kingdom and Senegal. J. Infect. Dis. 219, 1187–1197 (2019).
90. Frimpong, A. et al. Phenotypic evidence of T cell exhaustion and senescence during symptomatic Plasmodium falciparum malaria. Front. Immunol. 10, 1345 (2019).
91. Goronzy, J. J. & Weyand, C. M. Mechanisms underlying T cell ageing. Nat. Rev. Immunol. 19, 573–583 (2019).
92. Xu, M. et al. Senolytics improve physical function and increase lifespan in old age. Nat. Med. 24, 1246–1256 (2018).
93. Nagpal, N. et al. Small-molecule PAPD5 inhibitors restore telomerase activity in patient stem cells. Cell Stem Cell 26, 896–909 (2020).
94. Vukmanovic-Stejic, M. et al. Enhancement of cutaneous immunity during aging by blocking p38 mitogen-activated protein (MAP) kinase-induced inflammation. J. Allergy Clin. Immunol. 142, 844–856 (2018). This publication shows that the cutaneous response to varicella zoster virus is enhanced by treating elderly subjects with the oral p38 MAPK inhibitor losmapimod.
95. Walters, H. E., Deneka-Hannemann, S. & Cox, L. S. Reversal of phenotypes of cellular senescence by pan-mTOR inhibition. Aging 8, 231–244 (2016).
96. Mannick, J. B. et al. TORC1 inhibition enhances immune function and reduces infections in the elderly. Sci. Transl Med. 10, eaaq1564 (2018).
97. Finlay, C. M., Walsh, K. P. & Mills, K. H. Induction of regulatory cells by helminth parasites: exploitation for the treatment of inflammatory diseases. Immunol. Rev. 259, 206–230 (2014).
98. Maizels, R. M. & McSorley, H. J. Regulation of the host immune system by helminth parasites. J. Allergy Clin. Immunol. 138, 666–675 (2016).
99. Rosser, E. C. & Mauri, C. Regulatory B cells: origin, phenotype, and function. Immunity 42, 607–612 (2015).

100. Austermann, J., Roth, J. & Barczyk-Kahlert, K. The good and the bad: monocytes' and macrophages' diverse functions in inflammation. Cells 11, 1979 (2022).
101. Maizels, R. M. & Yazdanbakhsh, M. Immune regulation by helminth parasites: cellular and molecular mechanisms. Nat. Rev. Immunol. 3, 733–744 (2003).
102. Nguyen, D. X. & Ehrenstein, M. R. Anti-TNF drives regulatory T cell expansion by paradoxically promoting membrane TNF-TNF-RII binding in rheumatoid arthritis. J. Exp. Med. 213, 1241–1253 (2016).
103. Salgame, P., Yap, G. S. & Gause, W. C. Effect of helminth-induced immunity on infections with microbial pathogens. Nat. Immunol. 14, 1118–1126 (2013).
104. Kumar, N. P. et al. Strongyloides stercoralis coinfection is associated with greater disease severity, higher bacterial burden, and elevated plasma matrix metalloproteinases in pulmonary tuberculosis. J. Infect. Dis. 222, 1021–1026 (2020).
105. Wammes, L. J. et al. Regulatory T cells in human geohelminth infection suppress immune responses to BCG and Plasmodium falciparum. Eur. J. Immunol. 40, 437–442 (2010).
106. Van Braeckel-Budimir, N., Kurup, S. P. & Harty, J. T. Regulatory issues in immunity to liver and blood-stage malaria. Curr. Opin. Immunol. 42, 91–97 (2016).
107. Walther, M. et al. Upregulation of TGF-beta, FOXP3, and CD4+CD25+ regulatory T cells correlates with more rapid parasite growth in human malaria infection. Immunity 23, 287–296 (2005).
108. Nouatin, O. et al. Effect of immune regulatory pathways after immunization with GMZ2 malaria vaccine candidate in healthy lifelong malaria-exposed adults. Vaccine 38, 4263–4272 (2020).
109. Wait, L. F., Dobson, A. P. & Graham, A. L. Do parasite infections interfere with immunisation? A review and meta-analysis. Vaccine 38, 5582–5590 (2020).
110. Moncunill, G. et al. Distinct helper T cell type 1 and 2 responses associated with malaria protection and risk in RTS,S/AS01E vaccinees. Clin. Infect. Dis. 65, 746–755 (2017).
111. Nouatin, O. et al. Exploratory analysis of the effect of helminth infection on the immunogenicity and efficacy of the asexual blood-stage malaria vaccine candidate GMZ2. PLoS Negl. Trop. Dis. 15, e0009361 (2021).
112. Cooper, P. J. et al. Albendazole treatment of children with ascariasis enhances the vibriocidal antibody response to the live attenuated oral cholera vaccine CVD 103-HgR. J. Infect. Dis. 182, 1199–1206 (2000).
113. Webb, E. L. et al. Effect of single-dose anthelmintic treatment during pregnancy on an infant's response to immunisation and on susceptibility to infectious diseases in infancy: a randomised, double-blind, placebo-controlled trial. Lancet 377, 52–62 (2011).
114. Djuardi, Y., Wammes, L. J., Supali, T., Sartono, E. & Yazdanbakhsh, M. Immunological footprint: the development of a child's immune system in environments rich in microorganisms and parasites. Parasitology 138, 1508–1518 (2011).
115. Mpairwe, H., Tweyongyere, R. & Elliott, A. Pregnancy and helminth infections. Parasite Immunol. 36, 328–337 (2014).
116. Maizels, R. M., McSorley, H. J. & Smyth, D. J. Helminths in the hygiene hypothesis:

sooner or later? Clin. Exp. Immunol. 177, 38–46 (2014).

117. Barnes, P. J. Targeting cytokines to treat asthma and chronic obstructive pulmonary disease. Nat. Rev. Immunol. 18, 454–466 (2018).
118. Dees, S., Ganesan, R., Singh, S. & Grewal, I. S. Regulatory T cell targeting in cancer: emerging strategies in immunotherapy. Eur. J. Immunol. 51, 280–291 (2021).
119. Turner, V. M. & Mabbott, N. A. Influence of ageing on the microarchitecture of the spleen and lymph nodes. Biogerontology 18, 723–738 (2017).
120. Kityo, C. et al. Lymphoid tissue fibrosis is associated with impaired vaccine responses. J. Clin. Invest. 128, 2763–2773 (2018). This publication shows that in yellow fever vaccinees in Uganda, the extent of LN fibrosis can be linked to yellow fever vaccine responses.
121. Schacker, T. W. et al. Collagen deposition in HIV-1 infected lymphatic tissues and T cell homeostasis. J. Clin. Invest. 110, 1133–1139 (2002).
122. Ahmadi, O., McCall, J. L. & Stringer, M. D. Does senescence affect lymph node number and morphology? A systematic review. Anz. J. Surg. 83, 612–618 (2013).
123. Cakala-Jakimowicz, M., Kolodziej-Wojnar, P. & Puzianowska-Kuznicka, M. Aging-related cellular, structural and functional changes in the lymph nodes: a significant component of immunosenescence? An overview. Cells 10, 3148 (2021).
124. Lazuardi, L. et al. Age-related loss of naive T cells and dysregulation of T-cell/B-cell interactions in human lymph nodes. Immunology 114, 37–43 (2005).
125. Denton, A. E. et al. Targeting TLR4 during vaccination boosts MAdCAM-1+ lymphoid stromal cell activation and promotes the aged germinal center response. Sci. Immunol. 7, eabk0018 (2022).
126. Zou, Z., Lin, H., Li, M. & Lin, B. Tumor-associated macrophage polarization in the inflammatory tumor microenvironment. Front. Oncol. 13, 1103149 (2023).
127. Zou, W. P. Immunosuppressive networks in the tumour environment and their therapeutic relevance. Nat. Rev. Cancer 5, 263–274 (2005).
128. Pauken, K. E. & Wherry, E. J. Overcoming T cell exhaustion in infection and cancer. Trends Immunol. 36, 265–276 (2015).
129. Chodisetti, S. B. et al. Triggering through Toll-like receptor 2 limits chronically stimulated T-helper type 1 cells from undergoing exhaustion. J. Infect. Dis. 211, 486–496 (2015).
130. Ouyang, Q. et al. Bazedoxifene suppresses intracellular Mycobacterium tuberculosis growth by enhancing autophagy. mSphere 5, e00124-20 (2020).
131. Brodin, P. Immune-microbe interactions early in life: a determinant of health and disease long term. Science 376, 945–950 (2022).
132. Veldhoen, M. & Ferreira, C. Influence of nutrient-derived metabolites on lymphocyte immunity. Nat. Med. 21, 709–718 (2015).
133. Temba, G. S. et al. Urban living in healthy Tanzanians is associated with an inflammatory status driven by dietary and metabolic changes. Nat. Immunol. 22, 287–300 (2021). This publication shows that the difference in inflammatory profiles of individuals living in rural and urban areas of Tanzania might be attributed to food-derived metabolites in the diet.
134. Divangahi, M. et al. Trained immunity, tolerance, priming and differentiation: distinct immunological processes. Nat. Immunol. 22, 2–6 (2021).

135. Dominguez-Andres, J. et al. The itaconate pathway is a central regulatory node linking innate immune tolerance and trained immunity. Cell Metab. 29, 211–220 (2019).
136. Franco, F., Jaccard, A., Romero, P., Yu, Y. R. & Ho, P. C. Metabolic and epigenetic regulation of T-cell exhaustion. Nat. Metab. 2, 1001–1012 (2020).
137. Callender, L. A. et al. Mitochondrial mass governs the extent of human T cell senescence. Aging Cell 19, e13067 (2020).
138. O'Carroll, S. M. & O'Neill, L. A. J. Targeting immunometabolism to treat COVID-19. Immunother. Adv. 1, ltab013 (2021).
139. Li, S. et al. Metabolic phenotypes of response to vaccination in humans. Cell 169, 862–877.e17 (2017). This publication identifies several pathways involved in inositol phosphate or cholesterol metabolism that are linked to T and B cell responses to shingles vaccine (Zostavax).
140. Luo, W. et al. SREBP signaling is essential for effective B cell responses. Nat. Immunol. 24, 337–348 (2023).
141. DePeaux, K. & Delgoffe, G. M. Metabolic barriers to cancer immunotherapy. Nat. Rev. Immunol. 21, 785–797 (2021).
142. Patel, C. H., Leone, R. D., Horton, M. R. & Powell, J. D. Targeting metabolism to regulate immune responses in autoimmunity and cancer. Nat. Rev. Drug Discov. 18, 669–688 (2019).
143. Mannick, J. B. et al. mTOR inhibition improves immune function in the elderly. Sci. Transl Med. 6, 268ra179 (2014).
144. Kastenschmidt, J. M. et al. Influenza vaccine format mediates distinct cellular and antibody responses in human immune organoids. Immunity 56, 1910–1926.e7 (2023). This study shows that tonsil organoids can be used to compare responses to inactivated versus live attenuated influenza vaccine and gain an in-depth understanding of the differential adaptive immune responses that are elicited.
145. Turner, J. S. et al. SARS-CoV-2 mRNA vaccines induce persistent human germinal centre responses. Nature 596, 109–113 (2021).
146. Epstein, J. E. et al. Live attenuated malaria vaccine designed to protect through hepatic CD8+ T cell immunity. Science 334, 475–480 (2011).
147. Deroost, K. & Langhorne, J. Gamma/delta T cells and their role in protection against malaria. Front. Immunol. 9, 2973 (2018).
148. Black, G. F. et al. BCG-induced increase in interferon-gamma response to mycobacterial antigens and efficacy of BCG vaccination in Malawi and the UK: two randomised controlled studies. Lancet 359, 1393–1401 (2002).
149. van Riet, E. et al. Cellular and humoral responses to tetanus vaccination in Gabonese children. Vaccine 26, 3690–3695 (2008).
150. Roestenberg, M., Hoogerwerf, M. A., Ferreira, D. M., Mordmuller, B. & Yazdanbakhsh, M. Experimental infection of human volunteers. Lancet Infect. Dis. 18, e312–e322 (2018).
151. Gordon, S. B. et al. A framework for controlled human infection model (CHIM) studies in Malawi: report of a Wellcome Trust workshop on CHIM in low income countries held in Blantyre, Malawi. Wellcome Open Res. 2, 70 (2017).
152. Elliott, A. M. et al. Ethical and scientific considerations on the establishment of a controlled human infection model for schistosomiasis in Uganda: report of a stakeholders' meeting held in Entebbe, Uganda. AAS Open Res. 1, 2 (2018).

153. Chakaya, J. et al. Global Tuberculosis Report 2020 – reflections on the global TB burden, treatment and prevention efforts. Int. J. Infect. Dis. 113 (Suppl. 1), S7–S12 (2021).
154. Zwerling, A. et al. The BCG World Atlas: a database of global BCG vaccination policies and practices. PLoS Med. 8, e1001012 (2011).
155. Dockrell, H. M. & Smith, S. G. What have we learnt about BCG vaccination in the last 20 years? Front. Immunol. 8, 1134 (2017).
156. Abubakar, I. et al. Systematic review and meta-analysis of the current evidence on the duration of protection by bacillus Calmette–Guerin vaccination against tuberculosis. Health Technol. Assess. 17, 1–372 (2013).
157. WHO. Rotavirus vaccines: WHO position paper – July 2021. Wkly Epidemiol. Rec. 28, 301–320 (2021).
158. Vetter, V., Gardner, R. C., Debrus, S., Benninghoff, B. & Pereira, P. Established and new rotavirus vaccines: a comprehensive review for healthcare professionals. Hum. Vaccin. Immunother. 18, 1870395 (2022).
159. Plotkin, S. A. Recent updates on correlates of vaccine-induced protection. Front. Immunol. 13, 1081107 (2022).
160. Patel, M. et al. Oral rotavirus vaccines: how well will they work where they are needed most? J. Infect. Dis. 200, S39–S48 (2009).
161. Burnett, E., Parashar, U. D. & Tate, J. E. Real-world effectiveness of rotavirus vaccines, 2006–19: a literature review and meta-analysis. Lancet Glob. Health 8, e1195–e1202 (2020).
162. Bines, J. E. et al. Human neonatal rotavirus vaccine (RV3-BB) to target rotavirus from birth. N. Engl. J. Med. 378, 719–730 (2018).
163. Witte, D. et al. Neonatal rotavirus vaccine (RV3-BB) immunogenicity and safety in a neonatal and infant administration schedule in Malawi: a randomised, double-blind, four-arm parallel group dose-ranging study. Lancet Infect. Dis. 22, 668–678 (2022).
164. Cunliffe, N. A. et al. Efficacy of human rotavirus vaccine against severe gastroenteritis in Malawian children in the first two years of life: a randomized, double-blind, placebo controlled trial. Vaccine 30, A36–A43 (2012).
165. WHO. Vaccines and vaccination against yellow fever: WHO Position Paper, June 2013 — Recommendations. Wkly Epidemiol. Rec. 88, 269–283 (2015).
166. Barrett, A. D. & Teuwen, D. E. Yellow fever vaccine – how does it work and why do rare cases of serious adverse events take place? Curr. Opin. Immunol. 21, 308–313 (2009).
167. Pulendran, B. Learning immunology from the yellow fever vaccine: innate immunity to systems vaccinology. Nat. Rev. Immunol. 9, 741–747 (2009).
168. Jean, K., Donnelly, C. A., Ferguson, N. M. & Garske, T. A meta-analysis of serological response associated with yellow fever vaccination. Am. J. Trop. Med. Hyg. 95, 1435–1439 (2016).
169. WHO. Malaria vaccine: WHO position paper, March 2022. Wkly Epidemiol. Rec. 97, 61–80 (2022).
170. Olotu, A. et al. Efficacy of RTS,S/AS01E malaria vaccine and exploratory analysis on anti-circumsporozoite antibody titres and protection in children aged 5–17 months in Kenya and Tanzania: a randomised controlled trial. Lancet Infect. Dis. 11, 102–109 (2011).
171. Stoute, J. A. et al. A preliminary evaluation of a recombinant circumsporozoite protein vaccine against Plasmodium falciparum malaria. N. Engl. J. Med. 336, 86–91 (1997).
172. RTS,S Clinical Trials Partnership. Efficacy and safety of RTS,S/AS01 malaria vaccine with or without a booster dose in infants and children in Africa: final results of a phase 3,

individually randomised, controlled trial. Lancet 386, 31–45 (2015).

173. Richie, T. L. et al. Progress with Plasmodium falciparum sporozoite (PfSPZ)-based malaria vaccines. Vaccine 33, 7452–7461 (2015).
174. Roestenberg, M. et al. Protection against a malaria challenge by sporozoite inoculation. N. Engl. J. Med. 361, 468–477 (2009).
175. Elias, D., Britton, S., Aseffa, A., Engers, H. & Akuffo, H. Poor immunogenicity of BCG in helminth infected population is associated with increased in vitro TGF-β production. Vaccine 26, 3897–3902 (2008).
176. Nono, J. K., Kamdem, S. D., Musaigwa, F., Nnaji, C. A. & Brombacher, F. Influence of schistosomiasis on host vaccine responses. Trends Parasitol. 38, 67–79 (2022).
177. Hailegebriel, T. Undernutrition, intestinal parasitic infection and associated risk factors among selected primary school children in Bahir Dar, Ethiopia. BMC Infect. Dis. 18, 394 (2018).
178. Sweeny, A. R. et al. Supplemented nutrition decreases helminth burden and increases drug efficacy in a natural host-helminth system. Proc. Biol. Sci. 288, 20202722 (2021).
179. Martin, I. et al. The effect of gut microbiome composition on human immune responses: an exploration of interference by helminth infections. Front. Genet. 10, 1028 (2019).
180. Li, R. W. et al. The effect of helminth infection on the microbial composition and structure of the caprine abomasal microbiome. Sci. Rep. 6, 20606 (2016).
181. Walson, J. L. & Berkley, J. A. The impact of malnutrition on childhood infections. Curr. Opin. Infect. Dis. 31, 231–236 (2018).
182. Colgate, E. R. et al. Delayed dosing of oral rotavirus vaccine demonstrates decreased risk of rotavirus gastroenteritis associated with serum zinc: a randomized controlled trial. Clin. Infect. Dis. 63, 634–641 (2016).
183. Amaruddin, A. I. et al. BCG scar, socioeconomic and nutritional status: a study of newborns in urban area of Makassar, Indonesia. Trop. Med. Int. Health 24, 736–746 (2019).
184. Kim, A. H. et al. Enteric virome negatively affects seroconversion following oral rotavirus vaccination in a longitudinally sampled cohort of Ghanaian infants. Cell Host Microbe 30, 110–123.e5 (2022).
185. Hagan, T. et al. Antibiotics-driven gut microbiome perturbation alters immunity to vaccines in humans. Cell 178, 1313–1328.e13 (2019).
186. Allegretti, J. R., Mullish, B. H., Kelly, C. & Fischer, M. The evolution of the use of faecal microbiota transplantation and emerging therapeutic indications. Lancet 394, 420–431 (2019).
187. Li, S. et al. Molecular signatures of antibody responses derived from a systems biology study of five human vaccines. Nat. Immunol. 15, 195–204 (2014).
188. Kazmin, D. et al. Systems analysis of protective immune responses to RTS,S malaria vaccination in humans. Proc. Natl Acad. Sci. USA 114, 2425–2430 (2017).
189. Hagan, T. et al. Transcriptional atlas of the human immune response to 13 vaccines reveals a common predictor of vaccine-induced antibody responses. Nat. Immunol. 23, 1788–1798 (2022).
190. Tsang, J. S. et al. Improving vaccine-induced immunity: can baseline predict outcome? Trends Immunol. 41, 457–465 (2020).
191. Nakaya, H. I. et al. Systems analysis of immunity to influenza vaccination across multiple years and in diverse populations

reveals shared molecular signatures. Immunity 43, 1186–1198 (2015).

192. HIPC-CHI Signatures Project Team & HIPC-I Consortium. Multicohort analysis reveals baseline transcriptional predictors of influenza vaccination responses. Sci. Immunol. 2, eaal4656 (2017). By combining large data sets from influenza vaccine studies, this publication identifies a baseline predictive transcriptomic signature for influenza vaccine responses in young individuals, which did not apply to elderly individuals.
193. Kotliarov, Y. et al. Broad immune activation underlies shared set point signatures for vaccine responsiveness in healthy individuals and disease activity in patients with lupus. Nat. Med. 26, 618–629 (2020).
194. Fourati, S. et al. Pan-vaccine analysis reveals innate immune endotypes predictive of antibody responses to vaccination. Nat. Immunol. 23, 1777–1787 (2022).
195. Aiello, A. et al. Immunosenescence and its hallmarks: how to oppose aging strategically? A review of potential options for therapeutic intervention. Front. Immunol. 10, 2247 (2019).
196. Moncunill, G. et al. Antigen-stimulated PBMC transcriptional protective signatures for malaria immunization. Sci. Transl Med. 12, eaay8924 (2020).
197. Moncunill, G. et al. Transcriptional correlates of malaria in RTS,S/AS01-vaccinated African children: a matched case–control study. eLife 11, e70393 (2022). This publication shows that several baseline transcriptional signatures can associate with risk of malaria following vaccination with RTS,S.
198. Tsang, J. S. et al. Global analyses of human immune variation reveal baseline predictors of postvaccination responses. Cell 157, 499–513 (2014).

Chapter 3

Current status of schistosomiasis in school-aged children in mwanga district, Tanzania: Impact of two decades of annual mass drug administration programme.

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Abstract

Schistosomiasis is a neglected tropical disease with significant health implications, particularly among children. A cross-sectional study was conducted among school-aged children (SAC) in Mwanga district, Tanzania, a region known to be co-endemic for S. haematobium and S. mansoni infection and where annual mass drug administration (MDA) has been conducted for 20 years. In total, 576 SAC from 5 schools provided a urine sample for the detection of Schistosoma circulating anodic antigen using the upconverting particle-based lateral flow (UCP-LF CAA) test. Additionally, the potential of the point-of-care circulating cathodic antigen (POC-CCA) and microhaematuria dipstick test as field-applicable diagnostic alternatives for schistosomiasis were assessed and the prevalence outcome compared to UCP-LF CAA. Risk factors associated with schistosomiasis was assessed based on UCP-LF CAA. The UCP-LF CAA test revealed an overall schistosomiasis prevalence of 20.3%, compared to 65.3% based on a combination of POC-CCA and microhaematuria dipstick. No agreement was observed between the combined POC tests and UCP-LF CAA. Factors associated with schistosomiasis included age (5–10 years), involvement in fishing, farming, swimming activities and attending 2 of the 5 primary schools. Our findings suggest a significant progress in infection control in Mwanga district due to annual MDA, although not enough to interrupt transmission. Accurate diagnostics play a crucial role in monitoring intervention measures to effectively combat schistosomiasis.

Introduction

Schistosomiasis is a major neglected tropical disease disproportionately affecting sub-Saharan African countries, with 90.0% of the global disease burden occurring in this region[1]. In Tanzania, the overall prevalence of schistosomiasis is 51.5% [2], and among school-aged children (SAC) it is reported to be 53.5%[3], reaching up to about 80.0% in the northwestern zone around Lake Victoria[4,5]. However, current prevalence estimates do not include the northern region of Tanzania, including Mwanga district in the Kilimanjaro region. This district has been known to be endemic for both Schistosoma haematobium and Schistosoma mansoni [6]. The population is at high risk of schistosomiasis possibly due to the presence of the intermediate snail host (Bulinus and Biomphalaria) as well as irrigation schemes, which are the conducive environment for the transmission the Schistosoma spp. The presence of the hydroelectric dam known as 'Nyumba ya Mungu' (Fig. 1) which ensures a constant water supply to the surrounding villages for irrigation contributes to the continues transmission of schistosomiasis in Mwanga district [5]. In Tanzania, including the Kilimanjaro region, mass drug administration (MDA) of praziquantel has been the major strategy to reduce the burden of schistosomiasis and has been organized annually since 2004 among SAC who are at the highest risk of infection[7]. The need to assess the success of MDA has been highlighted by the World Health Organization and tools to enhance strategic guidance for schistosomiasis control program in Tanzania have equally been reported[8,9]. The most recent data on the prevalence status of schistosomiasis among SAC in Mwanga district are from 2005, indicating a prevalence ranging from 33.5 to 70.0%[6]. Conventional microscopy is the reference method to diagnose schistosomiasis in endemic settings and involves the detection of Schistosoma eggs in feces or urine, depending on the species. However, microscopy requires experienced, well-trained technical personnel, is considered a time-consuming, laborious method and has limited sensitivity in low-intensity infection settings[10]. Furthermore, the availability and access to microscopy is challenging in many rural areas in Tanzania due to a lack of trained personnel and appropriate infrastructure. Low-cost, user-friendly rapid tests could overcome such issues, but the accuracy of available point-of-care (POC) tests to determine the prevalence of schistosomiasis in regions co-endemic for S. haematobium and S. mansoni needs to be determined. The POC test for detecting Schistosoma Circulating Cathodic Antigen (POC-CCA) in urine has been endorsed by the WHO as an alternative to conventional microscopy, in particular for the diagnosis of S. mansoni infections[9]. It requires minimal training and has been validated in several field settings endemic for intestinal schistosomiasis[11, 12]. Another

easy-to-use rapid test is the microhaematuria dipstick test for the detection of haematuria, which has been shown to be strongly associated with urogenital schistosomiasis, although it is considered nonspecific[13]. A quantitative Up-Converting reporter Particle Lateral Flow (UCP-LF) test detecting the genus-specific Schistosoma Circulating Anodic Antigen (CAA) is a highly accurate test to detect active infection of all Schistosoma species in urine or serum[14]. [14]. Although it requires a more advanced laboratory infrastructure, it has been shown to be 100% specific and can reach a sensitivity to detect single-worm infections[14,15,16]. This study aimed to determine the current prevalence of schistosomiasis in the Mwanga district Tanzania after approximately twenty years of MDA using the UCP-LF CAA test and to explore the potential of using the POC-CCA and microhaematuria dipstick as a combined POC test for diagnosing schistosomiasis in co-endemic settings in comparison to the laboratory-based UCP-LF CAA test. Furthermore, we investigated Schistosoma infection risk factors and associated parameters.

Materials and methods

Ethical considerations

Ethical approval for this study was obtained from Kilimanjaro Christian Medical University College (KCMUCo) research and ethical committee board (reference number: 2588). Administrative authorization was obtained from the district education officer and Mwanga District Medical Officer. Children were enrolled based on their availability, and those willing to participate were given a consent form to be signed by guardians and/or parents. Immediately after sample collection all children, including those who participated in our study, were provided with praziquantel under the yearly MDA program at school at the recommended dose in the presence of a local clinician.

Study area and population

The study was conducted in Mwanga district, one of the seven districts of Kilimanjaro region in Tanzania. Farming, fishing, sand collection, pebble making, soil bricking and animal keeping are the major economic activities. The study was conducted in five schools, of which two were selected based on a previous study[6]. MDA of praziquantel had occurred in these schools more than 6 months prior to our study.

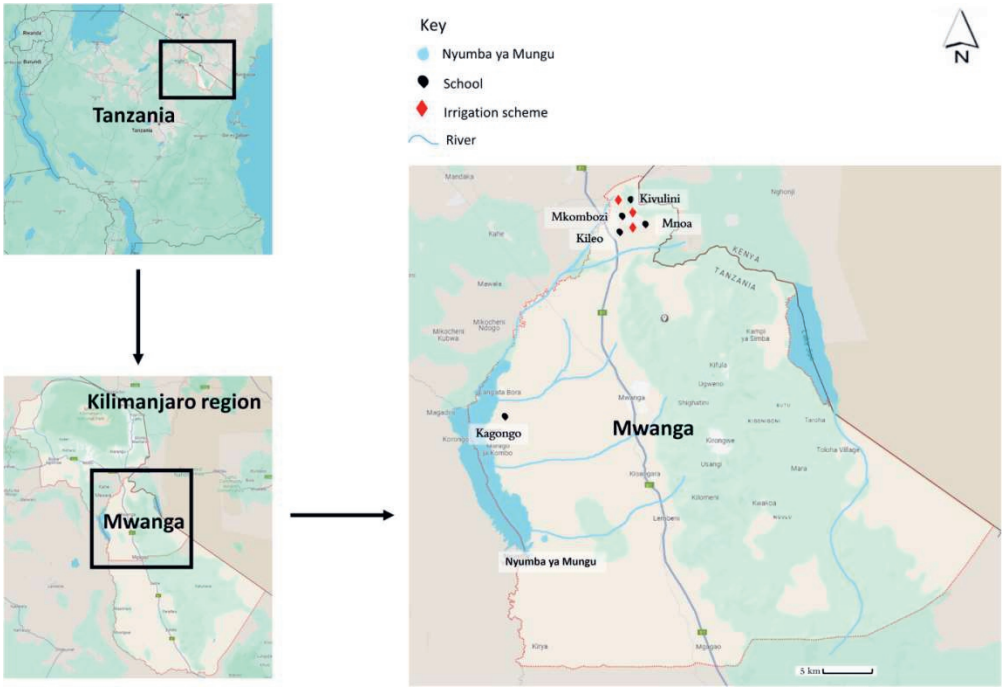

Figure 1. Map of Mwanga district Tanzania showing water sources, five primary schools and irrigation schemes

Sample/ data collection and processing

Enrolled study participants provided consent forms from parents and were given sterile containers with unique identifiers to provide fresh urine samples. For each sample, POC-CCA and microhaematuria dipstick was done in the field for the diagnosis of *S. mansoni* and *S. haematobium*, respectively. An aliquot of 2 mL of urine was conserved (at −20°C) per participant and was shipped on ice to Leiden University Medical Center in the Netherlands for retrospective UCP-LF CAA analysis. Following urine sample collection, a face-to-face interview using a closed-ended questionnaire in English and Swahili was conducted.

Field and laboratory analysis

The POC-CCA test (batch 180817091) was obtained from Rapid Medical Diagnostics, South Africa (SA), and analysis was done according to the manufacturer's instructions. Briefly, two drops of urine were transferred into the sample window of the test cassette. The readout of the cassette was done in 20 minutes. Results were scored as negative, trace or positive. Microhaematuria dipstick (Mission Urinalysis, Lot no: URS8090018) test was performed by

placing the strip on a flat surface and a drop of urine applied to the reagent pad. Readouts were done in 1 minute as either negative or positive according to the manufacturer's instructions.
The Schistosoma genus-specific UCP-LF CAA test was employed to detect CAA in urine samples and to confirm active infection with Schistosoma spp[14]. All urine samples were subjected to the UCAAhT417 wet format of the test. Briefly, 500 µL of each urine sample was mixed with 100 µL of 12% trichloroacetic acid, then incubated at room temperature for 5 minutes and centrifuged. The clear supernatant was then concentrated to 20 µL using a 0.5 mL centrifugal device (Amicon Ultra-0.5, Millipore, Merck Chemicals B.V., Amsterdam, The Netherlands). The resulting concentrate was then applied to the lateral flow test strip. To quantify CAA concentrations and to validate the assay cutoff (0.6 pg mL−1), reference standards with known CAA-levels were included. A CAA concentration above 0.6 pg mL−1 was considered positive. Infection intensity was categorized as low positive (>0.6–10 pg mL−1), moderate positive (>10–100 pg mL−1) and high positive (>100 pg mL−1).

Statistical analysis

The agreement between the combination of POC-CCA an microhaematuria dipstick (combined POC test) and UCP-LF CAA was performed using Kappa (K) statistics. For POC-CCA, trace results were considered negative. Furthermore, the association between socio-demographic characteristics and risk factors associated with *Schistosoma* infection, based on the UCP-LF CAA test, was performed using chi-square statistics, binary and multiple logistic regression analyses. Statistical analysis was performed using IBM Statistical Package for Social Sciences version 29 (SPSS Inc., Chicago, United States of America). For generation of plots, GraphPad Prism version 9.3.1 for Windows (GraphPad Software, San Diego, California USA, www.graphpad.com) was used.

Results

Socio-demographic characteristics

A total of 576 children provided informed consent and subsequently provided a urine sample and were therefore included in the final analysis. In Table 1 socio-demographic characteristics of the study population are given. The children's age ranged from 5 to 16 years, with a mean age of 9.8 years (**S . D .** 2.4) and 50.7% were females. Furthermore, the majority of the children were in class range 1 to 3 (50.3%). Farming was the most common father's profession

(46.7%) followed by fishing (23.4%). The most common mother's profession was farming (44.8%), followed by small businesses (34.2%).

Table 1. The prevalence of schistosomiasis across all five schools based on UCP-LF CAA, POC-CCA, microhaematuria dipstick and a combination of the POC-CCA and microhaematuria dipstick

			Diagnostic test			
			UCP-LF CAA	POC-CCA	Microheamaturia dipstick	Combined Test[a]
		Number of children	Positive (%)	Positive (%)	Positive (%)	Positive (%)
School	Kagongo	279	35 (12.5)	174 (62.4)	84 (30.1)	209 (74.9)
	Kileo	59	14 (23.7)	37 (62.7)	12 (20.3)	41 (69.5)
	Kivulini	57	19 (33.3)	30 (52.6)	12 (21.1)	36 (63.2)
	Mkombozi	106	31 (22.2)	35 (33.1)	23 (21.6)	43 (40.6)
	Mnoa	75	18 (24.0)	38 (50.6)	17 (22.7)	47 (62.7)
Total		**576**	**117 (20.3)**	**314 (54.5)**	**148 (25.7)**	**376 (65.3)**

[a] Combination of POC-CCA and/or microheamaturia dipstick positive outcome.

Prevalence and intensity of Schistosoma infection

In total 117 (20.3%) of children were found to be CAA positive (Table 1). The highest proportion of positives was observed among children attending Kivulini primary school (33.3%), followed by Mkombozi primary school (29.2%). The lowest proportion was 12.5% and was found among school children at Kagongo primary school. The majority of moderate to high intensity infections based on CAA-levels was observed in the schools in Kivulini and Mkombozi (Fig. 2a). Furthermore, SAC within the age category 5–10 years were found to be more often CAA positive than those aged 11–16 years (Fig. 2b). The outcome of the point-of-care tests are also summarized in Table 1. Based on POC-CCA and microhaematuria dipstick tests the prevalence of schistosomiasis were, 54.5% and 25.7% respectively. Assuming that the combination of two tests will give more clearer prevalence, combining POC-CCA and microhaematuria dipstick, the prevalence was 65.3%.

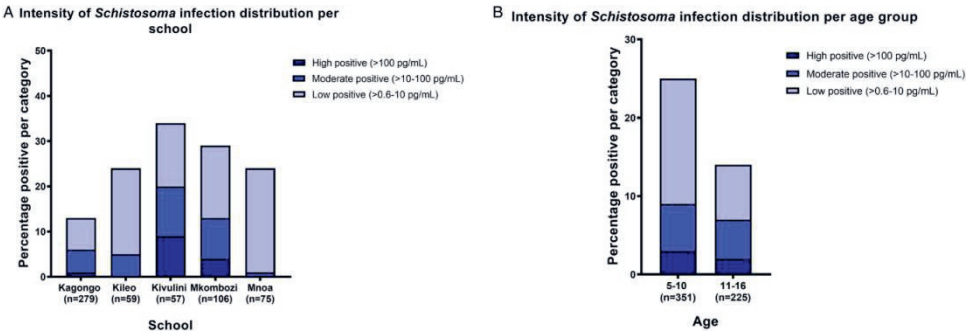

Figure 2. Prevalence and intensity of *Schistosoma* infection based on UCP-LF CAA amongst (A), five selected schools (B) the age categories.

The agreement between the combination of POC-CCA and microhaematuria dipstick and the reference test UCP-LF CAA

The combination of POC-CCA and microhaematuria dipstick showed no agreement with the UCP-LF CAA test (Table 2). Furthermore the *P* value indicates that this lack of agreement is not statistically significant, suggesting that the disagreement between tests is likely due to chance at significance level of 0.05. Analysis of the individual POC-CCA and microhaematuria dipstick tests also showed no agreement with the UCP-LF CAA test.

Table 2. The level of agreement between point-of-care circulating cathodic antigen (POC-CCA) test, microhaematuria dipstick and upconverting particle lateral flow circulating anodic antigen (UCP-LF CAA) urine test by Cohen's kappa coefficient in 576 school-aged children from Mwanga Tanzania

		UCP-LF CAA				
Test		Positive	Negative	*K*-value	*P* Value	Interpretation
POC-CCA	Positive	86	367	0.011	0.128	Poor
	Negative	31	92			
Microhaematuria dipstick	Positive	37	110	0.013	0.090	Poor
	Negative	80	349			
Combined (Microhaematuria and POC-CCA)	Positive	79	297	0.015	0.569	Poor
	Negative	38	162			

Risk factors associated with schistosomiasis

Using multivariate logistic regression analysis (adjusted odd ratio), the potential risk factors associated with *Schistosoma* infection, based on the presence of CAA, showed that children in class level 1–3 had two times higher odds of having schistosomiasis than children in higher classes. Children involved in farming and swimming activities had respectively 5.6 and 3.6

odds of being infected than those who did not farm nor swim. Furthermore, children attending Kileo, Kivulini, Mkombozi and Mnoa primary schools had 2.2, 2.6, 2.4 and 2.7 times higher odds of CAA positive results respectively, when compared to those attending Kagongo primary school. More details can be found in Supplementary Table 1.

Discussion

Using a highly accurate diagnostic approach (UCP-LF CAA), this study indicated that after nearly two decades of MDA schistosomiasis remains highly prevalent (20%) among school-aged children in Mwanga district, Tanzania. Although POC-CCA and microhaematuria dipstick test showed an even higher prevalence than the UCP-LF CAA test, no agreement was found between these tests and the UCP-LF CAA results nor any association was observed between these tests and known risk factors for schistosomiasis, highlighting the limitation of these currently available rapid diagnostics tests (POC-CCA and microhaematuria) in accurately determining the true prevalence in this specific setting that is known to be co-endemic for *S. mansoni* and *S. haematobium*.

Different prevalence's have been observed throughout Tanzania[17,18,19,20,21]. As commonly known as well as shown in the current study, measurement of prevalence highly depends on the diagnostic method used. Since we have used a highly accurate diagnostic method in our study, i.e. the UCP-LF CAA test, it is difficult to directly compare our results to previously published results based on microscopy and/or POC-CCA as these methods have limited sensitivity/ specificity. Our data confirm regional variation in the burden of schistosomiasis in Mwanga district, which would, extrapolated to Tanzania as a whole, argue for a more focally oriented schistosomiasis control approach. A significant difference in infection rates among different age groups was identified. Younger children (5–10 years) exhibited a higher prevalence of schistosomiasis than the older age group (11–16 years), indicating early exposure to the infection[20] . The possible reason for older children having low prevalence is through acquired immunity due repeated infections as indicated by other previous studies for example possible presence of IgE antibodies[22, 23]. A significant association was found between schistosomiasis and children who swim in water bodies, which may be attributed to playful behavioural activities common among children[24]. The children involved in swimming activities had 3.6 times more risk of being infected, in line with recent systematic review demonstrated by Reitzug and colleagues[25]. Children's involvement in farming was found to be associated with an increased risk of being infected with

schistosomiasis. Finally, children attending Kileo, Kivulini, Mkombozi and Mnoa primary schools were identified to have higher rates of Schistosoma infection compared to those attending Kagongo primary school. These findings are likely due to the proximity of irrigation schemes/rivers to these schools, which are perceived as safer water sources for young children and so most likely to visit compared to larger water bodies like the dams located closer to Kagongo, where parents have concerns about the risk of children drowning and so cautioned not to go there for water.

Despite of providing crucial updates on the prevalence of schistosomiasis among school children in the area, the study has several limitations. Firstly, the laboratory UCP-LF CAA test detects all Schistosoma species, but it does not provide any species-specific information[14]. In control settings, where species information would be relevant, other measures can provide this, e.g. determining the presence of specific snail species, or performing egg microscopy or species-specific PCR. For treatment, species information as such is not needed, and CAA has been demonstrated to be an excellent marker for monitoring treatment efficacy[26,16,27,28]. Although POC-CCA and microhaematuria rapid tests are user-friendly, kappa statistics revealed a poor agreement between these tests and the UCP-LF CAA. It was expected that the combined positivity rates of POC-CCA and microhaematuria dipstick test would reflect the UCP-LF CAA results, however this was not the case (Table 1). The poor agreement may be due to production batch differences, sexually transmitted infections (STIs), low-intensity infections, and subjectivity to test readouts, which might also affect results[29]. Furthermore, more accurate result with both tests might have been possible if the test was scored in a more quantitative manner. For example, following the recently described G-score scoring method for POC-CCA, an inclusion of control samples as a way to standardize the readout and to determine the cut-off for positivity. The microhaematuria test can be scored semi-quantitatively based on colour intensity linked to the level of red blood cells. However, registering of more quantitative results was not foreseen in this study.

In conclusion, this study demonstrates a moderate prevalence of schistosomiasis in Mwanga district Tanzania, implicating that the ~20 years annual MDA of praziquantel in this region may have had an effect on reducing the schistosomiasis burden, but transmission is still ongoing. To improve the efficacy of MDA strategies, diagnosis at acute stages of the disease in combination with treatment could be extended not only to higher risk groups but also to all persons above 2 years of age as recommended by WHO[30, 9]. Apart from that, integrated approaches including improved access to WASH infrastructure, political willingness, and

production of reliable data are important for controlling schistosomiasis in Tanzania[31]. The presence of persistent hotspots in countries like Tanzania where provision of MDA program failed to provide long terms solution in some villages shows the need for such an integrated approach[32]. Furthermore, a combination of the POC-CCA and microhaematuria dipstick did not prove to be useful as a screening tool for schistosomiasis in this S. haematobium and S. mansoni co-endemic setting. However, efforts are ongoing to make CAA detection generally available, with a recent initiative focusing on the development of a more easy-to-use, accurate, affordable and visually scored CAA-RDT[33,34]. The CAA-RDT could be of great potential in resource-poor endemic settings and assist in the development of targeted control measures and interventions to effectively combat schistosomiasis.

Supplementary material

The supplementary material for this article can be found at https://doi.org/10.1017/S0031182024001045.

Data availability statement

The authors confirm that the data supporting the findings of this study are available within the article and its supplementary and that the raw data is available upon request to the corresponding author.

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Authors' contributions

Conceptualization of the study: J.J.P, **S . E .** M, G.J.v.D, M.Y. Data collection, analysis interpretation of study findings: J.J.P, B.M, P.T.H, L.v.L, E.M, N.M, S.H, P.L.A.M.C, **S . E .** M, G.J.v.D, Editing: J.J.P, B.M, P.T.H, L.v.L, P.L.A.M.C, G.J.v.D; Original draft: J.J.P,B.M

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Competing interests

None.

Reference:

1. World Health Organization (WHO) (2023) Schistosomiasis facts 2023. Available at https://www.who.int/news-room/fact-sheets/detail/schistosomiasis. (accessed 1 July 2023).
2. Mazigo HD, Nuwaha F, Kinung'hi SM, Morona D, de Moira AP, Wilson S, Heukelbach J and Dunne DW (2012) Epidemiology and control of human schistosomiasis in Tanzania. Parasites & Vectors 5, 274.
3. Kavana NJ (2018) Prevalence of schistosomiasis infection among young children aged 5 to 17 years in Kilosa District, Tanzania: a 3 year retrospective review. Journal of Tropical Diseases 6, 255
4. Munisi DZ, Buza J, Mpolya EA and Kinung'hi SM (2016) Intestinal schistosomiasis among primary schoolchildren in two on-shore communities in Rorya district, northwestern Tanzania: prevalence, intensity of infection and associated risk factors.
   Journal of Parasitology Research 2016, 1–11.
5. Bakuza JS, Denwood MJ, Nkwengulila G and Mable BK (2017) Estimating the prevalence and intensity of Schistosoma mansoni infection among rural communities in Western Tanzania: the influence of sampling strategy and statistical approach. PLOS Neglected Tropical Diseases 11, e0005937.
6. Poggensee G, Krantz I, Nordin P, Mtweve S, Ahlberg B, Mosha G and Freudenthal S (2005) A six-year follow-up of schoolchildren for urinary and intestinal schistosomiasis and soil-transmitted helminthiasis in Northern Tanzania. Acta Tropica 93, 131–140.
7. Mwanga JR, Kinung'hi SM, Mosha J, Angelo T, Maganga J and Campbell CH (2020) Village response to mass drug administration for schistosomiasis in Mwanza Region, Northwestern Tanzania: are we missing socioeconomic, cultural, and political dimensions? The American Journal of Tropical Medicine and Hygiene 103, 1969–1977.
8. Clements ACA, Lwambo NJS, Blair L, Nyandindi U, Kaatano G, Kinung'hiS, Webster JP, Fenwick A and Brooker S (2006) Bayesian spatial analysis and disease mapping: tools to enhance planning and implementation of a schistosomiasis control programme in Tanzania. Tropical Medicine & International Health 11, 490–503..
9. World Health Organization (WHO) (2022) WHO guideline on Control and Elimination of Human Schistosomiasis. World Health Organization. Available at https://www.who.int/publications/i/item/9789240041608
10. Hoekstra PT, Madinga J, Lutumba P, van Grootveld R, Brienen EAT, Corstjens PLAM, van Dam GJ, Polman K and van Lieshout L (2022c) Diagnosis of schistosomiasis without a microscope: evaluating circulating antigen (CCA, CAA) and DNA detection methods on banked samples of a community-based survey from DR Congo. Tropical Medicine and Infectious Disease 7, 315.
11. Ochodo EA, Gopalakrishna G, Spek B, Reitsma JB, van Lieshout L, Polman K, Lamberton P, Bossuyt PM and Leeflang MM (2015) Circulating antigen tests and urine reagent strips for diagnosis of active schistosomiasis in endemic areas. Cochrane Database of Systematic Reviews 2015, CD009579.
12. Bärenbold O, Garba A, Colley DG, Fleming FM, Haggag AA, Ramzy RMR, Assaré RK,

Tukahebwa EM, Mbonigaba JB, Bucumi V, Kebede B, Yibi MS, Meité A, Coulibaly JT, N'Goran EK, Tchuem Tchuenté L-A, Mwinzi P, Utzinger J and Vounatsou P (2018) Translating preventive chemotherapy prevalence thresholds for Schistosoma mansoni from the Kato-Katz technique into the point-of-care circulating cathodic antigen diagnostic test. PLOS Neglected Tropical Diseases 12, e0006941.

13. Emukah E, Gutman J, Eguagie J, Miri ES, Yinkore P, Okocha N, Jibunor V, Nebe O, Nwoye AI and Richards FO (2012) Urine heme dipsticks are useful in monitoring the impact of Praziquantel treatment on Schistosoma haematobium in sentinel communities of Delta State, Nigeria. Acta Tropica 122, 126–131.
14. Corstjens PLAM, de Dood CJ, Knopp S, Clements MN, Ortu G, Umulisa I, Ruberanziza E, Wittmann U, Kariuki T, LoVerde P, Secor WE, Atkins L, Kinung'hi S, Binder S, Campbell CH, Colley DG and van Dam GJ (2020). Circulating anodic antigen (CAA): a highly sensitive diagnostic biomarker to detect active Schistosoma infections—improvement and use during SCORE. The American Journal of Tropical Medicine and Hygiene 103(1_Suppl), 50–57.
15. Langenberg MCC, Hoogerwerf M-A, Koopman JPR, Janse JJ, Kos-van Oosterhoud J, Feijt C, Jochems SP, de Dood CJ, van Schuijlenburg R, Ozir-Fazalalikhan A, Manurung MD, Sartono E, van der Beek MT, Winkel BMF, Verbeek-Menken PH, Stam KA, van Leeuwen FWB, Meij P, van Diepen A, van Lieshout L, van Dam GJ, Corstjens PLAM, Hokke CH, Yazdanbakhsh M, Visser LG and Roestenberg M (2020) A controlled human Schistosoma mansoni infection model to advance novel drugs, vaccines and diagnostics. Nature Medicine 26, 326–332.
16. Hoekstra PT, van Esbroeck M, de Dood CJ, Corstjens PLAM, Cnops L, van Zeijl-van der Ham CJG, Wammes LJ, van Dam GJ, Clerinx J and van Lieshout L (2021) Early diagnosis and follow-up of acute schistosomiasis in a cluster of infected Belgian travellers by detection of antibodies and circulating anodic antigen (CAA): a diagnostic evaluation study. Travel Medicine and Infectious Disease 41, 102053.
17. Mnkugwe RH, Minzi OS, Kinung'hi SM, Kamuhabwa AA and Aklillu E (2020) Prevalence and correlates of intestinal schistosomiasis infection among school-aged children in North-Western Tanzania. PLOS ONE 15, e0228770
18. Mazigo HD, Uisso C, Kazyoba P, Nshala A and Mwingira UJ (2021) Prevalence, infection intensity and geographical distribution of schistosomiasis among pre-school and school aged children in villages surrounding Lake Nyasa, Tanzania. Scientific Reports 11, 295.
19. Kajembe VR, Gasarasi DB, Tarimo DS, Lushina M and Sylvester B (2022) Prevalence and factors associated with persistent transmission of Schistosoma haematobium among primary school children after five rounds of mass drug administration using praziquantel: a cross sectional study in Mkuranga district, Tanzania. Tropical Doctor 52, 526–531.
20. Ogweno G, Mushi V, Silvestri V, Bonaventura W, Justine NC, Noah M, Yoram F, Mohamed H and Tarimo D (2023) Burden and risk factors for Schistosoma mansoni infection among primary school children: a quantitative school-based cross-sectional survey in Busega district, Northern Tanzania. PLOS ONE 18, e0280180.
21. Maganga JK, Campbell CH, Angelo T, Mosha J, Mwanga JR and Kinung'hi SM (2023) Test-Treat-Track-Test-Treat strategy for control of schistosomiasis in two low-prevalence villages in Northwestern Tanzania. The American

Journal of Tropical Medicine and Hygiene 108, 1167–1174.

22. Woolhouse MEJ (1998) Patterns in parasite epidemiology: the peak shift. Parasitology Today 14, 428–434.
23. Oettle R and Wilson S (2017) The interdependence between schistosome transmission and protective immunity. Tropical Medicine and Infectious Disease 2, 42.
24. Munisi DZ, Buza J, Mpolya EA, Angelo T and Kinung'hi SM (2017) Knowledge, attitude, and practices on intestinal schistosomiasis among primary schoolchildren in the Lake Victoria basin, Rorya District, northwestern Tanzania. BMC Public Health 17, 731.
25. Reitzug F, Ledien J and Chami GF (2023) Associations of water contact frequency, duration, and activities with schistosome infection risk: a systematic review and meta-analysis. PLOS Neglected Tropical Diseases 17, e0011377.
26. Sousa MS, van Dam GJ, Pinheiro MCC, de Dood CJ, Peralta JM, Peralta RHS, Daher E de F, Corstjens PLAM and Bezerra FSM (2019) Performance of an ultra-sensitive assay targeting the circulating anodic antigen (CAA) for detection of Schistosoma mansoni infection in a low endemic area in Brazil. Frontiers in Immunology 10, 682.
27. Hoekstra PT, Casacuberta-Partal M, van Lieshout L, Corstjens PLAM, Tsonaka R, Assaré RK, Silué KD, N'Goran EK, N'Gbesso YK, Brienen EAT, Roestenberg M, Knopp S, Utzinger J, Coulibaly JT and van Dam GJ (2022a) Limited efficacy of repeated praziquantel treatment in Schistosoma mansoni infections as revealed by highly accurate diagnostics, PCR and UCP-LF CAA (RePST trial). PLOS Neglected Tropical Diseases 16, e0011008.
28. Hoekstra PT, Chernet A, de Dood CJ, Brienen EAT, Corstjens PLAM, Labhardt ND, Nickel B, Wammes LJ, van Dam GJ, Neumayr A and van Lieshout L (2022b) Sensitive diagnosis and post-treatment follow-up of Schistosoma mansoni infections in asymptomatic Eritrean refugees by circulating anodic antigen detection and polymerase chain reaction. The American Journal of Tropical Medicine and Hygiene 106, 1240–1246.
29. Assaré RK, Tra-Bi MI, Coulibaly JT, Corstjens PLAM, Ouattara M, Hürlimann E, van Dam GJ, Utzinger J and N'Goran EK (2021). Accuracy of two circulating antigen tests for the diagnosis and surveillance of Schistosoma mansoni infection in low-endemicity settings of Côte d'Ivoire. The American Journal of Tropical Medicine and Hygiene 105, 677–683
30. Faust CL, Osakunor DNM, Downs JA, Kayuni S, Stothard JR, Lamberton PHL, Reinhard-Rupp J and Rollinson D (2020) Schistosomiasis control: leave no age group behind. Trends in Parasitology 36, 582–591.
31. Abe EM, Tambo E, Xue J, Xu J, Ekpo UF, Rollinson D, Yang K, Li S-Z and Zhou X-N (2020) Approaches in scaling up schistosomiasis intervention towards transmission elimination in Africa: leveraging from the Chinese experience and lessons. Acta Tropica 208, 105379.
32. Kittur N, King CH, Campbell CH, Kinung'hi S, Mwinzi PNM, Karanja DMS, N'Goran EK, Phillips AE, Gazzinelli-Guimaraes PH, Olsen A, Magnussen P, Secor WE, Montgomery SP, Utzinger J, Walker JW, Binder S and Colley DG (2019) Persistent hotspots in schistosomiasis consortium for operational research and evaluation studies for gaining and sustaining control of schistosomiasis after four years of mass drug administration of

praziquantel. The American Journal of Tropical Medicine and Hygiene 101, 617–627.

33. FIND (2020) Rapid test for precision mapping, and monitoring and evaluation of schistosomiasis control programmes. Available at https://www.finddx.org/what-we-do/projects/rapid-test-for-precision-mappingand-monitoring-and-evaluation-of-schistosomiasis-control-programmes/ (accessed 12 June 2024).
34. GHIT (2020) Schistosomiasis rapid diagnostic test to support control programmes in monitoring treatment impact and reassessment mapping. Available at https://www.ghitfund.org/ investment/portfoliodetail/detail/ 167/en (accessed 12 June 2024).

Chapter 4

Lifestyle score is associated with cellular immune profiles in healthy Tanzanian adults

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Abstract

Immune system and vaccine responses vary across geographical locations worldwide, not only between high and low-middle income countries (LMICs), but also between rural and urban populations within the same country. Lifestyle factors such as housing conditions, exposure to microorganisms and parasites and diet are associated with rural-and urban-living. However, the relationships between these lifestyle factors and immune profiles have not been mapped in detail. Here, we profiled the immune system of 100 healthy Tanzanians living across four rural/urban areas using mass cytometry. We developed a lifestyle score based on an individual's household assets, housing condition and recent dietary history and studied the association with cellular immune profiles. Seventeen out of 80 immune cell clusters were associated with living location or lifestyle score, with eight identifiable only using lifestyle score. Individuals with low lifestyle score, most of whom live in rural settings, showed higher frequencies of NK cells, plasmablasts, atypical memory B cells, T helper 2 cells, regulatory T cells and activated CD4+ T effector memory cells expressing CD38, HLA-DR and CTLA-4. In contrast, those with high lifestyle score, most of whom live in urban areas, showed a less activated state of the immune system illustrated by higher frequencies of naïve CD8+ T cells. Using an elastic net machine learning model, we identified cellular immune signatures most associated with lifestyle score. Assuming a link between these immune profiles and vaccine responses, these signatures may inform us on the cellular mechanisms underlying poor responses to vaccines but also reduced autoimmunity and allergies in low- and middle-income countries.

Introduction

Variation in the immune system have been observed across populations in low and middle-income countries (LMIC) in Africa and Asia and those living in high-income countries (HIC) in Europe and the USA[1-6]. In addition, immune system variation has been observed within countries, such as in rural compared to urban areas in Senegal[2], Tanzania[7] and Indonesia[1]. The immune system of rural-living individuals in LMICs shows higher memory, activated and regulatory immune profiles, characterized by among others regulatory T cells and T helper 2 cells (Th2 cells), compared to urban-living individuals[1, 2, 8, 9]. At the same time, reduced vaccine performance has been observed in populations living in LMICs, in particular in rural areas[4, 10, 11]. Moreover, it is known that in these same populations, there are less diseases of affluence, such as allergies or auto-immunities, where unchecked inflammation is a strong contributor[4, 11-19].

Several factors determine the immune profile of an individual, including genetic and demographic factors, such as age and sex, as well as environmental factors, including exposure to microorganisms and parasites, type of housing and dietary history[20, 21]. While genetics plays an important role in immune system variation during early childhood, this influence wanes with age due to cumulative exposure to environmental factors, including pathogens[20, 22, 23]. This has been illustrated in individuals chronically infected with helminths, who exhibit skewed baseline immune profiles, characterized by higher frequencies of Th2, regulatory T cells and higher expression of activation and inhibitory markers such as cytotoxic T lymphocyte-associated protein 4 (CTLA-4), HLA-DR and programmed cell death protein 1 (PD-1) on T cells[24-26]. Furthermore, individuals infected with cytomegalovirus (CMV) show a disproportionately higher activation state of the immune system and an increased frequency of memory cells[27, 28].

Socioeconomic status (SES) is intertwined with housing quality, nutritional status and access to healthcare[29, 30]. These factors contribute to infection risk and, therefore, propel the vicious circle of infection/infestation, which strongly impacts the immune system[18, 29-33]. The type of diet can also be linked to variation in immune profile, as was demonstrated in a recent study in Tanzania[7]. In this study, rural-living Tanzanians harbored a more anti-inflammatory immune profile that correlated with higher levels of plant-derived flavonoid apigenin found in food mostly eaten in rural settings[7]. Therefore, taken together, there is

evidence for links between living environments such as housing, exposure to microorganisms and parasites, SES including individual assets and diet and immune system variation in LMICs. Although the immune profiles of urban- and rural-living individuals have been directly compared, a more granular assessment of lifestyles irrespective of living location is lacking, as individuals living in rural areas may exhibit an urban lifestyle and vice versa. We hypothesized that a more refined measurement of lifestyle including housing status, assets (e.g. car, bicycle motorcycle or radio), and dietary history (i.e. frequency of consumption of common dietary products) will allow us to better explain immune variation previously related to rural or urban living location. Especially, we aim to more precisely define immune signatures in individuals exhibiting immune hypo-responsiveness. Such information can have an impact on both communicable and non-communicable diseases, as a poor immune response to vaccines will affect susceptibility to vaccine-preventable infections, while poor responses to (self-)antigens can lead to fewer allergies or autoimmune diseases in rural-living individuals. Therefore, we not only used mass cytometry to obtain a highly granular immune profile but also surveyed lifestyle variation among Tanzanian adults recruited from two rural and two urban locations to maximize lifestyle variation using a detailed questionnaire of housing conditions, assets and recent dietary history. We present a lifestyle score based on these questionnaire data, which places individuals on the spectrum ranging from rural to urban lifestyle. We used this lifestyle score to explain immune profile variation in Tanzanian adults living in rural and urban areas and contrasted this with immune signatures from urban-living Europeans. In addition, we utilized a machine learning model to define combined immune signatures most strongly associated with the lifestyle score.

Materials and Methods

Study design

This observational study was conducted between September and October 2022 as part of the CapTan study. A total of 203 healthy Tanzanian participants aged between 18 to 35 years were included from two urban locations (Urban Arusha and Urban Moshi) and two rural locations (Rural Moshi and Mwanga) in northern Tanzania (Figure 1A).

The study was approved both at a local level by the Ethical Board of the Kilimanjaro Christian Medical University College (No. 2588) and at the national level by the Tanzania National Ethical Committee Board (NIMR/HQ/R.8a/Vol.IX/4089). In addition, samples collected from

ten Dutch 18 to 30-year-old adults enrolled between January 2022 and September 2022 were included in the TINO study (ClinicalTrials.gov, reference no. NCT06039527). The study was approved by the Ethics Committee of Leiden University Medical Center (NL77841.058.21).

Description of study areas

Arusha City (1400m above sea level; 617,631 inhabitants[34]) is the administrative, business, commercial and educational centre of the Arusha region, as it accommodates most diplomatic and international activities. Due to these important regional functions, there is high diversity in ethnicity, economic status and lifestyle. Maasai, Meru and Chagga are the most common ethnicities. Most people living in Arusha City have access to good sanitation with the availability of clean, treated water. However, some people are slum dwellers, i.e. living in the city but practicing a rural lifestyle. Most people are self-employed or office employees in the government and private sectors[34].

Kilimanjaro region has about 1.9 million inhabitants[34] across seven different districts, three of which are included in this study (Moshi City, Rural Moshi and Mwanga). Moshi City (referred to as Urban Moshi) (700-950m above sea level; 331,733 inhabitants[34]) is the administrative, commercial and educational center of the Kilimanjaro region. Most people live a Western lifestyle and have good general sanitation and access to clean water. The main ethnicities are Chagga and Pare. Formal business is the main activity, followed by government and public employment, while few people are involved in agricultural and entrepreneurial activities[34].

People in Rural Moshi (535,803 inhabitants[34]) are mainly involved in agricultural activities. Some people have access to clean water, while few use borehole water sources. People live in large family units and their main economic activities are subsistence farming and animal husbandry. The main ethnicity is Chagga and people follow Chagga traditions, such as drinking local brew from banana/plantain.

The population of Mwanga district (684m above sea level; 148,763 inhabitants[34]) is mainly active in irrigation, subsistence farming and animal husbandry. The primary water sources are boreholes, rivers and dams, with only few people having access to tap water. Like Rural Moshi, people live in large family units. The main ethnicity is Pare, with few Chagga.

Europeans were recruited in the area around Leiden, an urban centre in The Netherlands. European individuals were Dutch.

Participant screening and enrollment

In rural communities, study information was given through community leaders and announcements during mass gatherings in mosques, churches and during village meetings. In urban communities, study information was distributed using leaflets and through community leaders, office announcements and university gatherings. Eligible participants (age 18-35 years and permanent residency of a given location) were asked to enroll in the study. Following informed consent, 230 participants were voluntarily screened for in- and exclusion criteria. Exclusion criteria were pregnancy, lactation, having acute or chronic diseases, being HIV-positive, recent use of antibiotics, use of antimalarials and use of tuberculostatic drugs. Participants were screened for HIV infection (SDBIOLINE HIV-1/2 3.0kit, LOT:03ADG020A), malaria (Malaria Ag p.f/Pan, Ref: 05FK60, LOT:05EDG018A) and soil-transmitted helminth such as hookworms (Ancylostoma duodenale and Necator americanus), Trichuris trichiura, Ascaris lumbricoides, Strongyloides stercoralis and Schistosoma mansoni using Kato-Katz or Schistosoma haematobium (POC-CCA, butch no:220701075). Furthermore, hemoglobin levels were measured (HemoCue Hb 301(CE:1450820055) and random blood glucose was assessed (ACCU-CHECK glucose test strips, Roche Diabetic care,06993761001). Weight and height were measured using a well-calibrated machine (RGZ-160, made from China), and last, blood pressure was measured using OMRON(SN:202111007949V). After nurse counseling, HIV-positive individuals who had low or high blood pressure (≤90/60mmHg and ≥140/90mmHg, respectively) or had high blood glucose (≥7.1mmol/L fasting or ≥11.1mmol/L random glucose) were excluded and guided for further actions. People diagnosed with schistosomiasis or soil-transmitted helminth infections were treated with praziquantel and albendazole, respectively according to Tanzanian treatment guidelines. Based on exclusion criteria, 27 of 230 participants were excluded.

All questionnaires and clinical samples were collected by a trained study team, consisting of medical doctors, nurses and laboratory scientists. Data from Tanzanian individuals were collected using the cloud-based electronic data collection system REDCap, with a server

hosted at the Kilimanjaro Clinical Research Institute in Tanzania. Data from Dutch participants were collected in a Castor database, with a server hosted in The Netherlands.

Lifestyle questionnaire

Questionnaires adopted from the Tanzania Demographic and Health Survey and Malaria Indicator Survey (TDHS-MIS) and previously published work conducted in Tanzania, focused on diet in relation to metabolic profiles and inflammatory status[7, 54] were used to collect data on basic demographics, wealth (house construction, general hygiene, land/animal/livestock/non-productive asset ownership) and (recent) food history. Combined, the collected information on wealth and food history was considered reflective of one's 'lifestyle'. Among others, our questionnaire included questions on the material used to construct the house's floor, roof and walls, the source of water, the type of toilet and available cooking facilities. We assessed the number of milk cows, cattle, goats, sheep, horses and poultry owned and inquiries were made on land ownership and possession of non-productive assets, such as radios, televisions, computers, refrigerators and ironing tools (whether powered by charcoal or electricity), watches, motorcycles, trucks, animal-drawn carts, generators and motorboats. As diet was recently found to shape immune responses in a Tanzanian population[7], we additionally collected data on recent food history. We specifically focused on the frequency of various food types participants consume per week, including ugali (stiff porridge), plantain, rice, potatoes, meat, fish, beans/peas, green vegetables, cabbage, fruits and local beer.

PBMC isolation and cryopreservation

Blood was collected in sodium heparin tubes from 189 of 203 participants. PBMC isolation and cryopreservation were performed as previously described[1]. 27 Samples were excluded due to low blood quality, technical problems during PBMC isolation or low cell counts. The remaining 162 cryopreserved PBMC samples were transported from Moshi, Tanzania, to Leiden, The Netherlands, using a liquid nitrogen dry vapor shipper. Out of these samples, we selected 100 individuals (25 per location) for immune phenotyping based on age, sex and educational level. Apart from these variables, baseline demographics for the total cohort and the mass cytometry cohort were comparable (Table 1 and Table S1).

Mass cytometry antibody staining

Antibody panels were designed to phenotype immune cells ex vivo. Details on antibodies used are listed in Table S4. Antibodies were conjugated to metal using 100µg of purified antibody combined with either the Maxpar X8 or MCP9 Antibody Labelling Kit (Fluidigm), as per the manufacturer's instructions. Conjugated antibodies were then stored in 200µl of Antibody Stabilizer PBS (CANDOR Bioscience GmbH) at 4°C. Titration of all antibodies was conducted on PBMC samples.

On the day of staining, cryopreserved PBMCs were thawed with 20% FCS/2mM Mg2+/1:10,000 benzonase/RPMI medium at 37°C and washed twice with 10% FCS/RPMI medium. For phenotyping, 3 × 106 cells per sample were prepared according to the Maxpar Nuclear Antigen Staining Protocol V2 (Fluidigm). PBMCs were washed with Maxpar staining buffer and centrifuged at 400g for 5 minutes in 5-ml Eppendorf tubes. Study samples were randomized over seven batches and for each batch up to 17 samples were barcoded. To barcode the samples, the cells were resuspended in 50µl of Maxpar staining buffer and 50µl of a barcode mix targeting β2-microglobulin (B2M) was added to each sample, employing a 6-choose-3 scheme using 106cadmium (Cd), 110Cd, 111Cd, 112Cd, 114Cd and 116Cd. After a 30-minute room temperature incubation and a wash with Maxpar Staining Buffer, the cells were centrifuged, the supernatant was removed and the cells were resuspended in Maxpar staining buffer and pooled into one tube for each batch.

Subsequently, cells were treated with 5ml (about 0.17 oz) of 500× diluted Cell-ID Intercalator-103Rh (Fluidigm) for 15 minutes to identify dead cells. After washing with staining buffer, cells were incubated with 20µl Human TruStain FcX Fc receptor blocking solution (BioLegend) and 130µl of staining buffer at room temperature for 5 minutes. Next, 150µl of a freshly prepared surface antibody cocktail was added for another 30-minute room-temperature incubation. After a double wash with staining buffer, cells were fixed with 1.6% PFA in 5ml PBS for 10 minutes. Post-centrifugation, cells underwent fixation and permeabilization using the eBioscience Foxp3/Transcription Factor Staining Buffer Set from eBioscience, followed by incubation with Human TruStain FcX receptor blocker. An intranuclear antibody cocktail was then added and the cells were incubated for an additional 30 minutes. After washing with permeabilization buffer and staining buffer, cells were fixed with 1.6% PFA in 5ml PBS for 10 minutes. Finally, cells are stained with 1000× diluted Cell-ID Intercalator-Ir (Fluidigm) in

Maxpar Fix and Perm Buffer at room temperature for 1h and stored in RPMI 20% FCS 10% DMSO at -80°C until acquisition.

Mass cytometry data acquisition

All barcoded samples within one batch were acquired simultaneously. Cells were measured using a Helios mass cytometer (Fluidigm) and calibrated as per Fluidigm's guidelines. Before measurement, cells underwent counting, washing with Milli-Q water, straining and then were suspended at a concentration of 1.0 × 106 cells/ml in a solution containing 10% EQ Four Element Calibration Beads from Fluidigm and Milli-Q water. Data acquisition in mass cytometry was performed using dual-count mode and with noise reduction. Various channels were used, including those for antibody detection, intercalators (103Rh, 191Ir, 193Ir), calibration beads (140Ce, 151Eu, 153Eu, 165Ho, 175Lu) and for tracking background/contamination (133Cs, 138Ba, 206Pb). Post-acquisition, the mass bead signal was used to standardize short-term signal variations, using the EQ passport P13H2302 as a reference throughout each experiment. When necessary, normalized FCS files were merged using Helios software, while retaining the beads.

Data analysis

All data preprocessing and statistics were performed in R v4.2.2 and RStudio Server v2022.03.999. All p-values were corrected for multiple testing using the Benjamini-Hochberg procedure (and referred to as q-values). P-/q-values<0.05 were considered statistically significant.

Data preprocessing

First, cells were automatically gated based on Gaussian parameters (CyTOFClean R-package; v1.03beta; https://github.com/JimboMahoney/cytofclean). Next, automatic gating was applied to select for intact/DNA+-(191Ir and 193Ir channels), CD45+- (89Y) and live cells (live/dead staining) (openCyto v2.10.1 R-package). All automatically set gates were manually inspected. Samples were compensated and debarcoded (CATALYST v1.22.0 R-package). Data were transformed using a hyperbolic arcsinh-transformation with a cofactor of 5 for downstream processing. Next, reference samples collected from healthy European adults included in each individual batch were used to train a CytoNorm-model (CytoNorm v0.0.17 R-package;

CytoNorm.train-function; nQ = 101; goal = ‘mean’; k = 10; limit = 0-8). The trained model was applied to all samples, adjusting for batch effects (CytoNorm.normalize-function).

Cell clustering

Cells were subjected to flowSOM-clustering (15 × 15 hexagonal grid; rlen=100; kohonen v3.0.11 R-package), followed by metaclustering at k = 80 clusters using the hierarchical clustering (factoextra v1.0.7 R-package, hcut-function, distance = ‘ward.D2’). The clustering map was trained on 100k cells per sample, the remaining cells were mapped to the trained map (predict.kohonen-function). Cell clusters were annotated at subset-level by an expert immunologist. Cell labels were further refined by incorporating markers that exhibit variability within a given subset in the cell label.

Lifestyle score

Multiple correspondence analysis (MCA) was applied to categorical questionnaire data (38 manually curated lifestyle-related questions; 21 on assets, 11 on food and 6 on housing) for all 203 Tanzanian participants (FactoMineR v2.7 R-package, MCA-function). Missing values are imputed using mode imputation. Principle component (PC) 1 was defined as ‘lifestyle score’, as this component, per definition, explained most variance across lifestyle questionnaire data. Coordinates of samples and variable categories were visualized in biplots. In addition, (cumulative) variable category contributions for lifestyle score were extracted and shown.

Statistical analyses

To understand the overall structure of the data, cells were placed on a two-dimensional t-distributed Stochastic Neighbor Embedding (t-SNE) map using the Fit-SNE algorithm v1.2.1 (https://github.com/KlugerLab/Fit-SNE/blob/master/fast_tsne.R). Fit-SNE was performed on a down-sampled dataset including 1,500 cells per sample (max_iter = 1,000; learning rate = n cells/12; perplexity = n cells/100).

To compare the frequency of cell clusters across rural and urban Tanzanian locations, we employed a generalized linear mixed model (binomial = ‘family’; link = ‘logit’; lme4 R-package v1.1-31). The number of cells in each cell cluster (as a fraction of total CD45+ cells per sample) was considered the dependent variable. We fit two models to assess the overall effect of location. Model 1 included (scaled) age and sex as fixed explanatory variables and

'sample ID' as a random intercept. 'Sample ID' was included as a random effect to deal with any under- or overdispersion due to the binomial model. Model 2 was the same as model 1, except that 'location' was added as a fixed explanatory variable. ANOVA tests were used to assess whether location (model 2) significantly improved model fit compared to model 1. Significant models (after correction for multiple testing using Benjamini-Hochberg) were subjected to pairwise comparisons between locations using the emmeans v1.8.5 R-package (Tukey post hoc test). The associations between cell cluster frequency and lifestyle score were also assessed using GLMMs, including lifestyle score, (scaled) age and sex as fixed explanatory variables and 'sample ID' as a random intercept. For sensitivity analyses, we fitted an additional 'combined' GLMM, including both location and lifestyle (LS) (as well as age (scaled) and sex) as fixed effects and sample ID as random effect. Model fit (using Akaike Information Criterion [AIC]) of the 'combined' GLMM was compared to same model, after removing either location or lifestyle score, to assess the relative importance of these variables to performance cluster-specific models.

Elastic net machine learning modelling

To identify a combined immune 'endotype' most associated with variation in lifestyle score, we fit an elastic net machine learning model (tidymodels v1.1.1 R-package, glmnet-engine). Scaled age, sex and cell frequencies of all 80 clusters were included as predictors and lifestyle score was included as an outcome variable. Data was randomly split into train (80%) and test (20%) data (stratified for living location). Model tuning was performed on training data using 2,000 bootstrapped data samples, optimizing penalty and mixture parameters. The best model was identified based on the highest explained variance (R2) between observed and predicted lifestyle score (penalty = 0.788, mixture = 0.1). The final model was applied to both training and testing data to generate final estimates of model fit (R2). Variable importance was assessed using the vip v0.4.1 R-package. Feature stability was assessed by extracting all features from the models fitted with the optimized tuning parameters across bootstrap datasets (n = 2,000). The number of times a feature was selected was used as a measure for feature stability.

Results

Characteristics of the study population

The Tanzanian study population consisted of 203 adults recruited from four geographical locations in northern Tanzania, including two urban locations, Arusha and Moshi Urban and

two rural locations, Moshi Rural and Mwanga (Figure 1A). These four locations were categorized as rural and urban based on the National Bureau of Statistics and the 2022 Census[34]. Detailed information on housing, assets and food history was collected using questionnaires[7, 35] (Figure 1B).

From these 203 individuals (Table S1), PBMC samples of 100 individuals were included for mass cytometry analyses (n = 100; n = 25 from each site in four sites) (Table 1). The median age was 25.0 years (interquartile range [IQR], 23-29 years). The prevalence of parasitic infections was 7% and these infections were detected only in individuals from rural areas (Table 1). As a comparator cohort, PBMC samples from ten Dutch individuals recruited in Leiden, The Netherlands (median age 29 [IQR 27-30], 50% female) were acquired using mass cytometry (referred to as 'urban European').

Table 1 | Baseline characteristics of the study population (*N* = 100).

Variable	Overall, N = 100	Urban Arusha, N = 25	Urban Moshi, N = 25	Rural Moshi, N = 25	Rural Mwanga, N = 25	p-value
Sex, female	53 (53%)	14 (56%)	14 (56%)	13 (52%)	12 (48%)	0.932
Age	25.0 (23.0, 29.0)	25.0 (23.0, 30.0)	25.0 (24.0, 27.0)	24.0 (22.0, 27.0)	25.0 (22.0, 31.0)	0.686
Age categories						0.955
18-25	56 (56%)	13 (52%)	14 (56%)	15 (60%)	14 (56%)	
26-36	44 (44%)	12 (48%)	11 (44%)	10 (40%)	11 (44%)	
BMI	22.8 (20.5, 26.0)	21.8 (19.0, 26.8)	24.1 (22.9, 28.4)	22.3 (20.3, 26.7)	22.4 (21.3, 24.6)	0.243
Missing	1	1	0	0	0	
BMI classification						0.591
<18.5	7 (7.1%)	3 (13%)	2 (8.0%)	1 (4.0%)	1 (4.0%)	
18.5-24.9	60 (61%)	14 (58%)	13 (52%)	15 (60%)	18 (72%)	
25.0-29.9	16 (16%)	2 (8.3%)	5 (20%)	4 (16%)	5 (20%)	
>30	16 (16%)	5 (21%)	5 (20%)	5 (20%)	1 (4.0%)	
Missing	1	1	0	0	0	
Systolic blood pressure (mmHg)	119 (110, 125)	110 (109, 120)	110 (100, 119)	121 (112, 130)	123 (119, 128)	<0.001
Missing	1	1	0	0	0	
Diastolic blood pressure (mmHg)	73 (70, 79)	70 (70, 77)	69 (64, 72)	78 (70, 80)	78 (74, 80)	<0.001
Missing	1	1	0	0	0	

Hemoglobin level g/dl	14.35 (13.30, 16.50)	14.00 (13.30, 16.60)	13.80 (12.40, 15.60)	14.20 (13.70, 16.00)	15.20 (13.80, 16.60)	0.223
Random blood sugar, mmol-1^^	5.20 (4.60, 5.95)	4.90 (4.40, 5.50)	5.20 (4.70, 6.23)	5.20 (4.10, 5.50)	5.80 (4.90, 6.50)	0.053
Missing	1	0	1	0	0	
Highest level of education						<0.001
Primary	30 (30%)	0 (0%)	0 (0%)	13 (52%)	17 (68%)	
Secondary	24 (24%)	6 (24%)	0 (0%)	10 (40%)	8 (32%)	
College	15 (15%)	12 (48%)	1 (4.0%)	2 (8.0%)	0 (0%)	
University	31 (31%)	7 (28%)	24 (96%)	0 (0%)	0 (0%)	
Malaria	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	
Missing	1	0	1	0	0	
Helminth infection[a]	7 (7.0%)	0 (0%)	0 (0%)	2 (8.0%)	5 (20%)	0.015
Schistosomiasis[b]	3 (3.0%)	0 (0%)	0 (0%)	0 (0%)	3 (12%)	0.057
Missing	1	1	0	0	0	
Insurance status	31 (31%)	13 (52%)	15 (60%)	3 (12%)	0 (0%)	<0.001
Occupation						<0.001
Farming	20 (20%)	0 (0%)	1 (4.0%)	5 (20%)	14 (56%)	
Elementary occupation	28 (28%)	5 (20%)	2 (8.0%)	16 (64%)	5 (20%)	
Student	23 (23%)	5 (20%)	15 (60%)	2 (8.0%)	1 (4.0%)	
Employed/business owner	20 (20%)	10 (40%)	5 (20%)	2 (8.0%)	3 (12%)	
Not employed	9 (9.0%)	5 (20%)	2 (8.0%)	0 (0%)	2 (8.0%)	

N = 100 participants. Values represent number of participants (percentage of total) and median (interquartile range [IQR]) for categorical and continuous variables, respectively. Comparisons between locations were performed using Fisher's exact, chi-squared and Mann–Whitney U-test for categorical and continuous variables, respectively. [a] Stool was tested for helminths using the Kato-Katz method, testing for *Schistosoma haematobium*, *Schistosoma mansoni*, *Ascaris Lumbricoides*, hookworm and *Trichuris trichuria*. [b] Tested for schistosomiasis using the POC-CCA method, testing for *Schistosoma haematobium* and *Schistosoma mansoni*.

Cellular immune profiles differ between rural- and urban-living Tanzanian adults.

To characterize the cellular immune profiles between rural- and urban-living individuals, peripheral blood mononuclear cells (PBMCs) were stained with a panel of 37 metal-tagged antibodies. The processed single-cell level dataset contained 69.6 million live CD45+ cells, which allowed the identification of six major immune lineages, including B cells, CD4+ T cells, CD8+ T cells, innate lymphoid cells (ILCs), myeloid cells and unconventional T cells (including γδ T cells) (Figure 1C). Clustering analyses using self-organizing maps (SOM), followed by hierarchical clustering resulted in 80 distinct immune cell clusters (Figure S1 and Table S2). Cell clusters were annotated at subset-level by an expert immunologist. Cell labels were further refined by incorporating markers that exhibit variability within a given subset in the cell label. Using Generalized Linear Mixed Models (GLMMs), we identified nine clusters which were significantly different between the four locations, after adjusting for age and sex (Figure 1D-E).

The CD4+ T cell lineage was composed of 28 cell clusters, of which 5 significantly differed across locations. Th2 cells (cluster 51) represented the strongest rural signal, where we observed significantly higher frequencies in rural-living locations (especially rural Moshi) compared to urban-living individuals (median 0.7% of total CD45+ cells across rural sites compared to 0.3% and 0.2% in urban Tanzanians and Europeans, respectively). Rural-living individuals additionally showed a significantly higher frequencies of three cell clusters of CD4+ T cells. These clusters included CD161dim PD-1dim CTLA-4+ CD4+ T effector memory (Tem) cells (cluster 46), CD4+ Tem cells expressing CD38, CD161, CTLA-4 and PD-1 (cluster 79) and HLA-DRdim PD-1+ KLRG-1+ CD4+ Tem cells (cluster 72). In contrast, the CD27+ CD28+ CD45RO+ CD127+ CD4+ T central memory (Tcm) cell cluster (cluster 53) was higher in urban compared to rural-living individuals (Figure 1E).

Within the CD8+ T cell lineage, 1 out of 15 CD8+ T cell clusters significantly differed across locations. This cluster was characterized by recently activated CD8+ Tem cells expressing CXCR3 and T-bet (cluster 11), which showed higher frequencies in urban compared to both rural locations (Figure 1E). Furthermore, within the gamma delta (γδ) T cell lineage (containing 7 clusters), naïve γδ T cells expressing CXCR3 (cluster 40) were significantly higher in frequency in urban living compared to both rural-living individuals. Finally, within the B cell lineage, we observed significantly higher frequencies of classical naive B cells

(cluster 34) and atypical memory B cells expressing CD11c and Tbet (cluster 35) in rural- compared to urban-living locations (Figure 1E). Six out of seven rural-associated clusters showed visual evidence of a rural-urban-European gradient, where cell frequencies showed a stepwise decrease from rural-to-urban and urban-to-European sites, except for cluster 40 (naïve γδ T cells). On the other hand, gradients were less clear for clusters enriched in urban Tanzanians.

Questionnaire data reveal differences in lifestyle between locations.

Within living locations, considerable variation in immune signatures was observed. Therefore, to better capture immune variation across locations, we developed a lifestyle score, which incorporates detailed questionnaire data on assets (e.g. possession of a watch, television or car), housing (i.e. materials used to construct the house) and food history (i.e. frequency of consumption of dietary products) into a single score. To obtain the lifestyle score, we applied Multiple Correspondence Analysis (MCA), a dimensionality reduction method similar to Principal Component Analysis (PCA), but for categorical data, which was applied to 38 questions (118 variable categories) collected from all 203 participants (Table S3 and Figure S2). MCA clearly separated individuals based on living location, especially across principal component (PC) 1. Since the MCA was based on lifestyle questionnaire data and PC1 per definition explains most variance, PC1 was referred to as 'lifestyle score', explaining 7.8% of the variation in the questionnaire data (Figure 2A). Across the first two principal components, we found that spread was highest in rural- compared to urban-living individuals (variance 6.1%/5.1% and 11.3%/11.2% for PC1/PC2 scores across urban and rural sites, respectively), indicating rural people have more heterogeneous lifestyles (Figure 2B). Sensitivity analyses on condensed questionnaire data (collapsing rare categories and removing uninformative variables) showed that the relatively low percentage of variance explained by lifestyle score and other high-ranking principle components (Figure S3A) is caused by the inclusion of rarer variable categories. Removing these had no important effect on the lifestyle score (Pearson r = 0.97, p-value < 2.2 × 10-16).

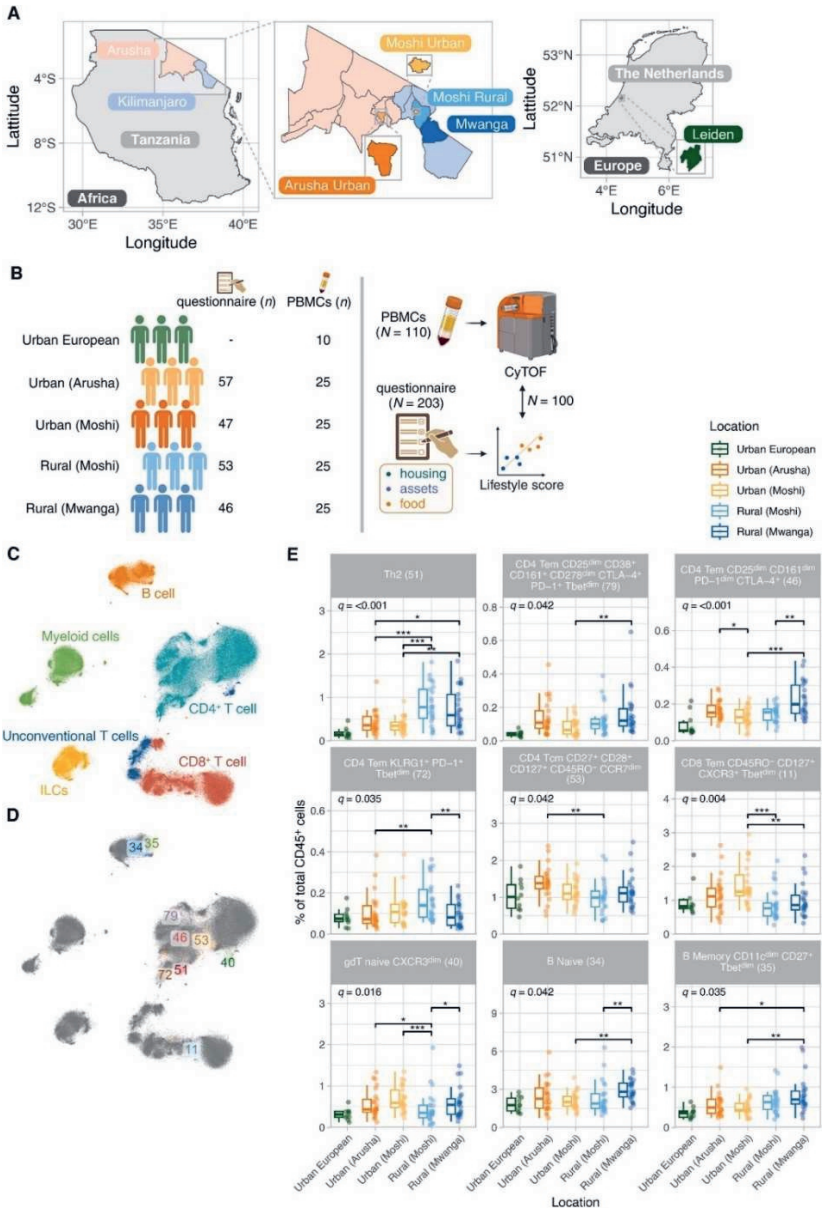

Figure 1 | Mass cytometry immune profiles differ across individuals living in rural (Moshi Rural and Mwanga) and urban (Arusha and Moshi Urban) regions.

A) Map of study sites in Tanzania and in The Netherlands. B) Graphical representation of sample numbers and the study design. C-D) t-distributed Stochastic Neighbor Embedding (t-SNE) visualizations (n = 1500 random cells/individual); cells are coloured according to lineage (C) or significant cell cluster (D). E) Differential cell frequencies between rural and urban Tanzanian regions. Boxplots represent the 25th and 75th percentiles (lower and upper boundaries of boxes, respectively), the median (middle horizontal line) and measurements that fall within 1.5 times the interquartile range (IQR; distance between 25th and 75th percentiles; whiskers). Only clusters showing a

significant effect of 'location' (across Tanzanian sites) were shown. The significance of 'location' was assessed using analysis of variance (ANOVA)-tests comparing a full (location, age [scaled] and sex [fixed effects] and sample ID [random effect]) and a simpler model, which was the same as the full model, except that we removed 'location' from the model. ANOVA p-values were corrected for multiple testing using the Benjamini-Hochberg method and referred to as q-values. Asterisks denote statistical significance (*, $q \leq 0.05$; **, $q \leq 0.01$; ***, $q \leq 0.001$). The statistical significance of differences between each location was assessed using the emmeans()-function (Tukey post hoc test). Urban Europeans were included in the figure for visual comparisons and were not included in statistical tests.

We found that the lifestyle score was significantly associated with thirteen of 80 cell clusters, while none of the other principal components (PC2-PC5) showed any statistically significant associations with cell cluster frequencies (Figure S3B), underscoring the validity and biological relevance of the lifestyle score.

Next, we explored the most strongly contributing lifestyle score variables across questionnaire categories, including housing conditions, assets and food history. Overall, assets showed the highest cumulative contribution to the lifestyle score (53.6%), followed by housing (30.3%) and food variables (16.1%) (Figure 2D). Among the top 20 variables most strongly contributing to PC1, factors such as having a house with an earth/sand floor, a mud wall, no household electricity and a pit latrine as toilet were associated with low lifestyle score. Additionally, the lack of assets such as an ironing tool, refrigerator, computer, radio, car, television, or watch and not consuming potatoes was associated with a low lifestyle score. Factors associated with a high lifestyle score were a house with a flush toilet connected to a sewage/septic tank, a separate room used as a kitchen and possessing assets such as a car, a working computer and a refrigerator (Figure 2E).

Besides lifestyle score (PC1), we found that PC2 explained 4.1% of the variance (Figure S3A) and showed the highest spread across individuals living in rural Mwanga (variance across PC2 scores 15.0% compared to 2.9%-7.0% in other sites) (Figure 2B). Similar to PC1, variables related to assets were most important (cumulative contribution 66.0%), particularly those related to livestock farming (Figure S3C). PC3 through PC5 explained 3.2-3.5% of the variance (Figure S3A), generally showing a higher cumulative contribution of food variables (40.3-49.4%) (Figure S3C) compared to PC1 and PC2.

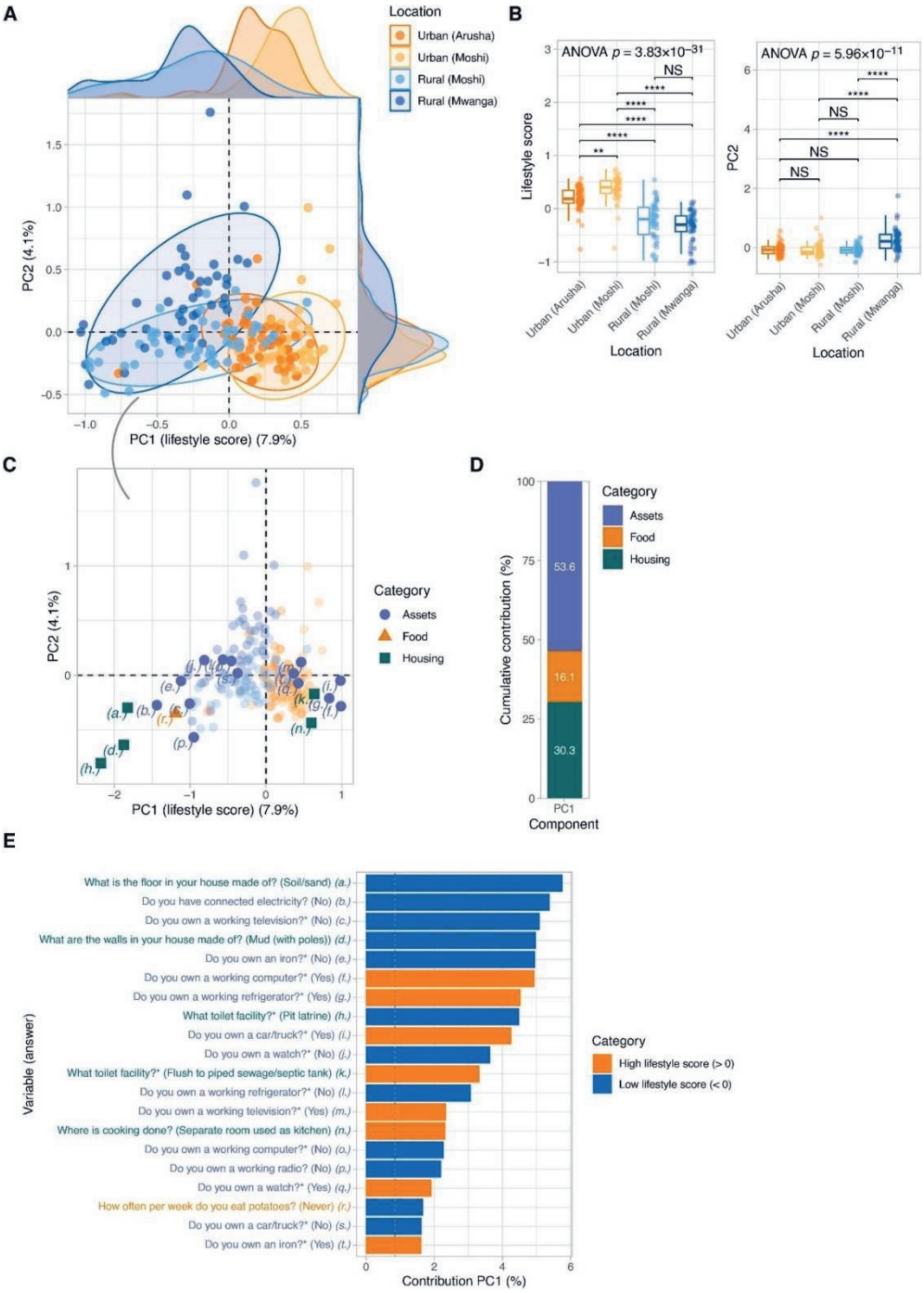

Figure 2 | Multiple Correspondence Analysis (MCA) based on questionnaire data to generate lifestyle score.
A) MCA was applied to categorical questionnaire data (38 manually curated questions; 21 on assets, 11 on food and 6 on housing) (N = 203 individuals). Data points are coloured based on location. Ellipses reflect the data spread at a

level of confidence of 95%. Density plots show the distribution of PC1 (lifestyle score) (x-axis) and PC2 (y-axis) score. B) Comparisons of PC1 (lifestyle score) and PC2 across locations. Global significance was assessed using analysis of variance (ANOVA) and *post hoc* tests between locations were performed using Tukey HSD tests. Asterisks denote statistical significance (NS, non-significant; *, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.001$, $p \leq 0.0001$). C) Coordinates of each variable category (a.-t.; see E) across dimensions 1 and 2. Variable categories with similar profiles are grouped together. D) Cumulative contributions (in percentage) of the variable categories by questionnaire data category (i.e. housing, assets and food). E) Contributions (in percentage) of variable categories to PC1 or lifestyle score. Bars are coloured based on whether a variable was associated with a high (> zero) or low (< zero) lifestyle score.

Lifestyle score association tests reveal additional immune cell clusters not previously linked to living location

We next assessed the association between lifestyle score and immune cell frequencies using GLMMs, adjusting for age and sex. We first verified that lifestyle score in individuals with matching mass cytometry data (n = 100), which was not significantly different from individuals without mass cytometry data available (Figure S4).

Overall, 13 cell clusters were associated with lifestyle score, of which 8 clusters were not identified by previous analyses where we assessed differences in immune profile between locations (Figure 3A and 3B). Indeed, only one of these clusters (cluster 12; CD8+ naïve) showed a trend towards significance across locations ($q = 0.055$; Figure S5). In addition, we confirmed 5 out of 9 clusters which were previously found to significantly differ across locations, which were Th2 cells (cluster 51; GLMM; $\beta = -0.66$), two CD4+ Tem clusters that were CTLA-4+ and/or CD161+ (cluster 79 and 46; $\beta = -0.50$ and -0.28, respectively), atypical memory B cells (cluster 35; $\beta = -0.37$) (rural-living location and low lifestyle score) and a CD8+ Tem cluster (cluster 11; $\beta = 0.32$) (urban-living location and high lifestyle score) (Figure 3C). The additional clusters identified using the lifestyle score were two CD4+ Tem cell clusters that were associated with low lifestyle score: HLA-DR+ PD-1+ CD4+ Tem (cluster 43; $\beta = -0.38$) and regulatory T cells (cluster 75; $\beta = -0.35$). Furthermore, we identified a cluster of plasmablasts (cluster 57; $\beta = -0.49$), which was enriched in those with low lifestyle score. Last, an innate immune cell cluster of NK-cells (cluster 25; $\beta = -0.68$) was also linked to a low lifestyle score (Figure 3D).

In contrast, within the CD8+ T cell lineage, we identified three clusters of CD8+ T cells that were associated with high lifestyle score. These included two CD8+ naïve T cell clusters (cluster 12 and 21; $\beta = 0.38$ and 0.39, respectively) and a cluster of CD8+ Tem cells expressing

CD161 and KLRG1 (cluster 38; β = 0.59). In addition, we found a positive association between higher frequencies of ILC2 (cluster 60; β = 0.33) and a high lifestyle score (Figure 3D). Sensitivity analyses, where we jointly modelled lifestyle score and location and compared the model fit to simpler models (excluding either lifestyle score or location), indicated that indeed using lifestyle score we can detect an additional group of clusters which we could not have detected with location alone (Figure S6).

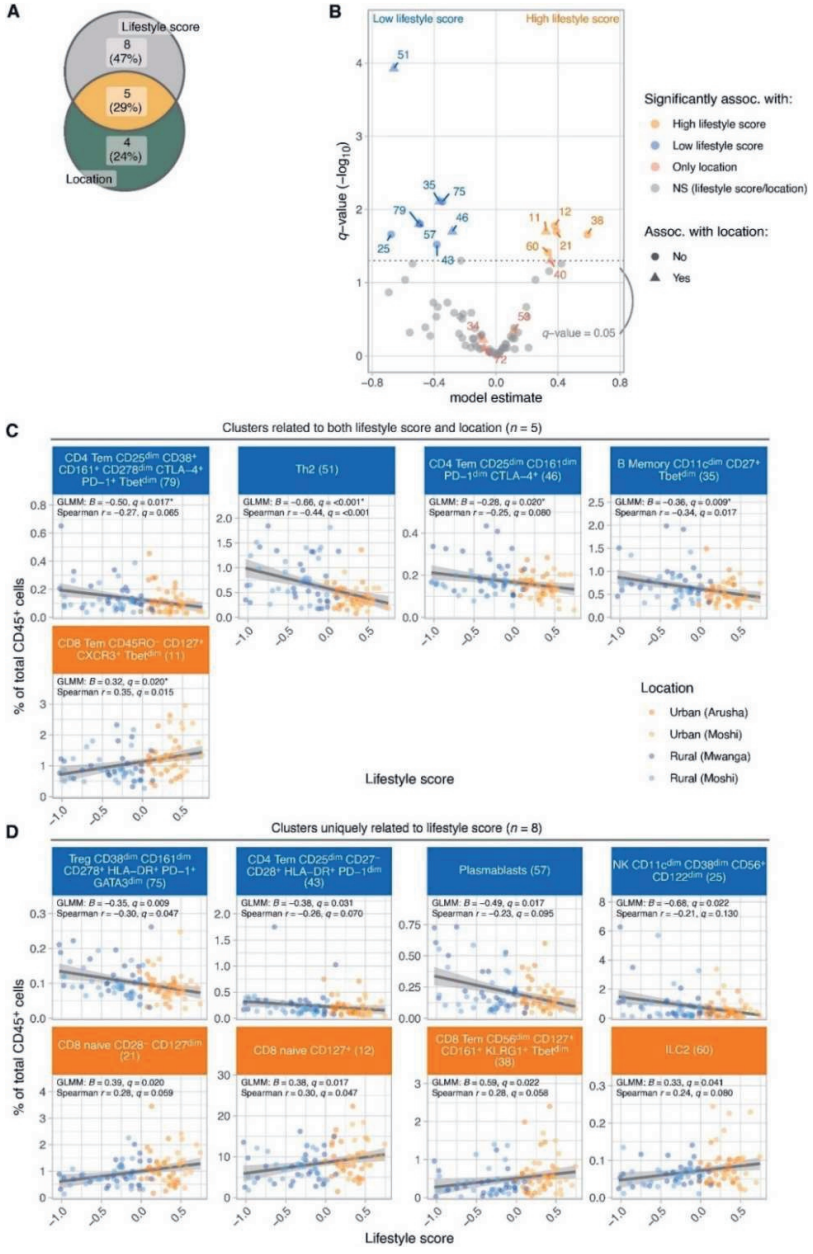

Figure 3 | Lifestyle score is associated with specific immune cell clusters not identified by comparisons across locations.

A) Venn diagram indicating the number of cell clusters that show differences in cell frequencies 1) across locations [Figure 1E], 2) both across locations and lifestyle score [Figure 3C] and 3) only with lifestyle score [Figure 3D]. Eight cell clusters were uniquely associated with lifestyle score and were not identified by comparisons across sampling locations. B) Volcano plot showing differential frequency results. Results were derived from a GLMM with cell frequency as outcome variable, lifestyle score, age (scaled) and sex as fixed effects and sample ID as a random effect. Model estimates and corresponding Benjamini-Hochberg (BH)-adjusted p-values ($-\log_{10}$(q-value)) were shown. Each point represents a cluster, clusters with q-values<0.05 are coloured by association (high or low lifestyle score, or only significantly associated with location). Shapes indicate whether lifestyle-associated clusters were also detected by comparisons across sampling locations. Each point is labelled with a cluster identifier. C-D) Scatter plots showing the association between lifestyle score and cell frequency for C) clusters significantly related to both location as well as lifestyle score and D) clusters uniquely related to lifestyle score (i.e. clusters not identified as differentially abundant between locations). Data points are coloured based on location. Lines represent linear fits to the data and are included for visualization purposes only. Statistical significance was assessed using a linear mixed model including lifestyle score, age (scaled) and sex as fixed effects and sample ID as random effect. Additionally, we ran univariable Spearman correlation tests, p-values were corrected for multiple testing using the Benjamini-Hochberg method (q-value). Asterisks indicate clusters that significantly differed between locations. Only cell clusters significant in GLMMs are shown.

Machine learning modelling links a combined immune endotype with a lifestyle score

To investigate if a combination of immune cell clusters could be identified that together is associated with a lifestyle score ('immune endotype'), a machine learning model (elastic net) was trained with lifestyle score as an outcome and cell cluster frequencies, age and sex as the predictor variables. Model training and hyperparameter tuning were performed on 80% of the data (n = 80 individuals; 2,000 bootstrapped datasets) and the model was tested on the remaining 20% of the data (n = 20 individuals) (Figure 4A). The model was able to predict 44.1% and 29.6% of the variance in the training and test data, respectively. Using feature importance analysis, we verified 11 of the 14 clusters that were previously associated with living location and/or lifestyle score. Compared to previous analyses, the current model is a multivariable model, estimating the contribution of each cell cluster to the prediction of lifestyle score while adjusting for all other cluster cell frequencies. Therefore, using this complementary approach, we identified three additional clusters, including CD8+ Tem cells expressing CD161 and KLRG1 (cluster 37) associated with high lifestyles score, pDCs (cluster 58) and γδ T-cells (cluster 22) related to low lifestyle score (Figure 4B).

Taken together the elastic net model unveiled a fairly stable (Figure 4C) immune endotype characterized by Th2 cells, regulatory T cells, atypical B memory cells, plasmablasts, NK, CTLA-4+ CD161+ CD4+ Tem, KLRG1+ γδ T-cells and plasmacytoid dendritic cells (pDCs) associated with a low lifestyle score. Inversely, the immune profile characterized by CD8+ naïve T cells, CXCR3+ CD127+ CD8+ Tem, two CD8+ Tem CD161+ CD56dim KLRG1+ and ILC2 is associated with a high lifestyle score (Figure 4B).

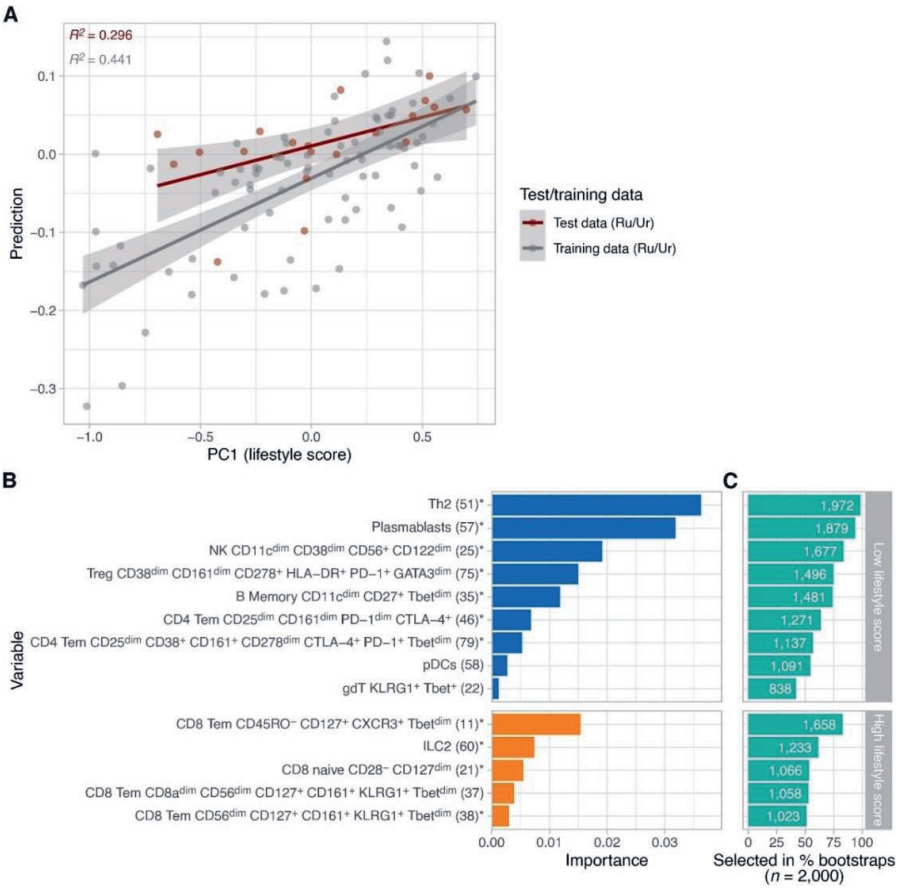

Figure 4 | Machine learning model based on cell cluster frequencies can partly reconstruct lifestyle score.
A) Performance of an elastic net machine learning model based on cell cluster frequencies (n = 80), age and sex trained to predict lifestyle score. Observed compared to predicted lifestyle score based on training (80%) and test data (20%; n = 5 samples per location) are shown. Using cell frequency data, we can explain ~30% of the variance in lifestyle scores (leave-out test data). B) Feature importance of all features that remained in the model after feature shrinkage/regularization. Clusters previously associated with either location or lifestyle score (n = 17) are indicated (*). Three clusters have not been associated with location nor lifestyle score in previous analyses. C) Feature stability across bootstraps. All features from the models fitted with the optimized tuning parameters (penalty/mixture) were extracted. The number of times a feature was selected across bootstrap samples serves as a score for stability of that feature (maximum score = 2,000).

Discussion

Here, we assessed the associations between location and/or lifestyle score and cellular immune profiles measured by mass cytometry. We found that seventeen of 80 clusters were associated with location or lifestyle score, with eight identifiable only when using lifestyle score, illustrating the ability of lifestyle score to capture immune variation. Indeed, individuals living in rural areas may exhibit an urban lifestyle and vice versa. This was further substantiated by applying a machine learning model, which identified a combined immune signature associated with lifestyle score.

We found an association between low lifestyle score and expression of activation markers such as CD38, HLA-DR and CTLA-4 on CD4+ Tem cells, along with expansion of Th2 and an increased frequency of regulatory T cells expressing CTLA-4. An increase in a specific memory T cell subsets might indicate that fewer naïve T cells are available for activation and expansion upon encounter with a new antigen. Furthermore, expression of activation/inhibitory markers on T cells can result in a reduced response to vaccines and allergens but may also explain a lower prevalence of autoimmune diseases in LMICs[19, 24, 36]. Indeed, in rural Senegalese, immune profiles were enriched for HLA-DR-expressing CD4+ T cells compared to urban-living individuals[2]. Previous studies comparing rural and urban populations in Indonesia[1, 25] and Gabon[26, 37] found that immune profiles in rural-living individuals, characterized by high frequencies of Th2 cells, T regulatory cells expressing CTLA-4, HLA-DR, ICOS or CD161 and atypical memory B cells, were strongly linked to (chronic) helminth infections[1, 25, 26].

In contrast to these previous studies, none of our participants tested positive for malaria and the prevalence of current helminth infections was very low. Therefore, we speculate that increased activation of CD4+ Tem cells, along with expansion of Th2 and higher regulatory T cell frequencies, may represent an immune footprint left behind by parasitic infection in the past or even during childhood, as have been suggested by others [24, 38, 39]. Indeed, in 2005, the prevalence of schistosomiasis among school-aged children in two different schools located in one of the rural areas included in this study ranged between 34-70% with evidence for the presence of other soil-transmitted infections in the same setting[40]. Thus, based on their age, our study participants likely experienced a high burden of helminth infections during childhood.

Alternatively, housing conditions related to a low lifestyle score (e.g. sand or earth floors and mud-wall houses) may predispose to different commensals or exposure to bacteria and fungi and their metabolites[41], some of which have immunomodulatory properties. Poor housing conditions also attract vectors like flies, lice, ticks, mites and mosquitoes, which may directly activate the immune system through components present in their saliva, even in the absence of disease transmission[31, 42]. Furthermore, rural-living individuals closely live with livestock and as such are exposed to an additional reservoir of micro-organisms and (zoonotic) pathogens[43]. Taken together, past (parasitic) infections or unmeasured variables, such as the microbiome or exposure to vectors, are tightly linked to housing conditions. These factors may drive lifestyle-related immune variation, resulting in enrichment of Th2, regulatory T cells and activated T cells.

We found that individuals with low lifestyle score most of whom live in rural settings, display a higher frequency of plasmablasts. Plasmablasts are differentiated B cells with a short lifespan, which initiate early antibody responses during infections [44-46]. However, due to their high metabolic activity, the rapid development of short-lived plasmablasts can paradoxically impair humoral immunity by slowing down germinal centre formation. This, in turn may impair responsiveness to vaccines and reduce risk of developing allergies and autoimmunity by limiting the generation of long-lived plasma and memory B cells. Although this has been shown in the context of malaria infection [47], which is not endemic in northern Tanzania, other infectious diseases endemic in the area, may similarly induce high levels of plasmablasts, including dengue[48].

Last, we identified an association between both naïve CD8+ T cells and CD8+ Tem expressing CD161 and high lifestyle score. Although we lack immune markers to confirm, CD161+CD8+ Tem encompasses mucosal-associated invariant T cells (MAIT) cells. MAIT cells are abundant in blood and at mucosal sites and can activate dendritic cells that promote T follicular helper cells to induce mucosal antigen-specific IgA[49]. Therefore, the presence of such cells in urban-living individuals might indicate the propensity to react more strongly to antigens in a vaccine, allergens, or autoantigens. This aligns with the results of an earlier study indicating that healthy individuals residing in urban Moshi had a higher pro-inflammatory cytokine response upon pathogen challenge in an ex vivo PBMC stimulation assay compared to those living in rural areas[7, 35]. Regarding the naïve CD8+ T cells being enriched in urban living,

it has been noted that they allow new immune responses to be mounted to both infections and vaccines[50]. Their higher frequency in urban areas is in line with previous studies in Bangladeshi compared to (urban living) North American children within the first three years of life[51] as well as in Malawian compared to UK adults[52]. Reduced numbers of naïve CD8+ T cells was associated with a higher burden of intestinal worms and viral infections (e.g. CMV) in children from Bangladesh compared to those from the USA[3] and higher burden of CMV among Malawian adults[52]. Similarly, we speculate that the association between high life score and naive CD8+ T cells in our study is driven by reduced pathogen exposure in people living in urban settings due to differences in daily activities and hygiene practices compared to rural-living individuals.

The strengths of this study include the use of mass cytometry data in combination with the availability of detailed information on housing, assets and food history. Condensing this information into a single score allowed us to train a machine learning model to identify a distinct group of cell clusters (termed 'immune endotype'), which was strongly associated with lifestyle score variation. Previous studies in HICs indicated that baseline (gene-expression-based) immune endotypes exhibiting a strong pro-inflammatory profile are predictive of improved vaccine responses in young adults across multiple vaccines[53]. In a similar fashion, we speculate the immune endotypes identified in this study are linked to vaccine responses in populations living in rural or urban Africa. As such, further phenotyping of immune endotypes in varied populations, not limited to HIC, using protein-based single-cell modalities such as mass cytometry, may deepen our understanding of variation in vaccine responses or reactivity to allergens or autoantigens and their underlying mechanisms. At the same time, using lifestyle scores opens opportunities for public health experts to screen individuals prone to, for example, vaccine hypo-responsiveness, informing policymakers on preventative measures, such as repeated vaccination. These interventions could target these high-risk individuals, potentially improving vaccine efficacy and public health outcomes. Since those mounting reduced vaccine responses are the very same individuals that also show lower responses to allergens and auto-antigens, immune phenotyping may also unveil new ways to prevent non-communicable diseases in urban-living individuals. Our study also has limitations. Among others, we did not assess cellular immune function through stimulation assays. In addition, future studies establishing direct links between low lifestyle score and responses to vaccines, allergens and autoantigens would be of great value.

In conclusion, in this study we comprehensively assessed the association between immune profiles and location and lifestyle variables in a LMIC. Additional cell clusters were detected through a more refined measurement of lifestyle. Follow-up studies should therefore focus on the links between lifestyle score, immune signature and functional immune responses, particularly in populations where vaccine responses are expected to be reduced and in populations with the highest prevalence of diseases linked to exaggerated immune responses to allergens and autoantigen.

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References

1. de Ruiter, K., et al., Helminth infections drive heterogeneity in human type 2 and regulatory cells. Science Translational Medicine, 2020. 12(524).
2. Mbow, M., et al., Changes in immunological profile as a function of urbanization and lifestyle. Immunology, 2014. 143(4): p. 569-577.
3. Wager, L.E., et al., Increased T Cell Differentiation and Cytolytic Function in Bangladeshi Compared to American Children. Frontiers in Immunology, 2019. 10.
4. Muyanja, E., et al., Immune activation alters cellular and humoral responses to yellow fever 17D vaccine (vol 124, pg 3147, 2014). Journal of Clinical Investigation, 2014. 124(10): p. 4669-4669.
5. Smolen, K.K., et al., Pattern recognition receptor-mediated cytokine response in infants across 4 continents. Journal of Allergy and Clinical Immunology, 2014. 133(3): p. 818-+.
6. de Jong, S.E., et al., Systems analysis and controlled malaria infection in Europeans and Africans elucidate naturally acquired immunity. Nature Immunology, 2021. 22(5): p. 654-+.
7. Temba, G.S., et al., Urban living in healthy Tanzanians is associated with an inflammatory status driven by dietary and metabolic changes. Nature Immunology, 2021. 22(3): p. 287-+.
8. Anuradha, R., et al., Parasite Antigen-Specific Regulation of Th1, Th2, and Th17 Responses in Infection. Journal of Immunology, 2015. 195(5): p. 2241-22500.
9. Kemp, K., B.D. Akanmori, and L. Hviid, West African donors have high percentages of activated cytokine producing T cells that are prone to apoptosis. Clinical and Experimental Immunology, 2001. 126(1): p. 69-75.
10. Domingo, C., et al., Long-term immunity against yellow fever in children vaccinated during infancy: a longitudinal cohort study. Lancet Infectious Diseases, 2019. 19(12): p. 1363-1370.
11. van Dorst, M., et al., Immunological factors linked to geographical variation in vaccine responses. Nat Rev Immunol, 2023.
12. Tsang, J.S., et al., Improving Vaccine-Induced Immunity: Can Baseline Predict Outcome? Trends in Immunology, 2020. 41(6): p. 457-465.
13. Nehar-Belaid, D., et al., Baseline immune states (BIS) associated with vaccine responsiveness and factors that shape the BIS. Seminars in Immunology, 2023. 70.
14. Shannon, C.P., et al., Multi-Omic Data Integration Allows Baseline Immune Signatures to Predict Hepatitis B Vaccine Response in a Small Cohort. Frontiers in Immunology, 2020. 11.
15. Tsang, J.S., et al., Global Analyses of Human Immune Variation Reveal Baseline Predictors of Postvaccination Responses (vol 157, pg 499, 2014). Cell, 2014. 158(1): p. 226-226.
16. Avey, S., et al., Multicohort analysis reveals baseline transcriptional predictors

of influenza vaccination responses. Science Immunology, 2017. 2(14).

17. Okada, H., et al., The 'hygiene hypothesis' for autoimmune and allergic diseases: an update. Clinical and Experimental Immunology, 2010. 160(1): p. 1-9.
18. Murdaca, G., et al., Hygiene hypothesis and autoimmune diseases: A narrative review of clinical evidences and mechanisms. Autoimmunity Reviews, 2021. 20(7).
19. Bach, J.F., Mechanisms of disease: The effect of infections on susceptibility to autoimmune and allergic diseases. New England Journal of Medicine, 2002. 347(12): p. 911-920.
20. .Brodin, P., et al., Variation in the human immune system is largely driven by non-heritable influences. Cell, 2015. 160(1-2): p. 37-47.
21. Klein, S.L. and K.L. Flanagan, Sex differences in immune responses. Nat Rev Immunol, 2016. 16(10): p. 626-38
22. .Brodin, P. and M.M. Davis, Human immune system variation. Nat Rev Immunol, 2017. 17(1): p. 21-29.
23. Liston, A., et al., Human immune diversity: from evolution to modernity. Nat Immunol, 2021. 22(12): p. 1479-1489.
24. Lubyayi, L., et al., Infection-exposure in infancy is associated with reduced allergy-related disease in later childhood in a Ugandan cohort. Elife, 2021. 10.
25. Wammes, L.J., et al., Community deworming alleviates geohelminth-induced immune hyporesponsiveness. Proceedings of the National Academy of Sciences of the United States of America, 2016. 113(44): p. 12526-12531.
26. Labuda, L.A., et al., A Praziquantel Treatment Study of Immune and Transcriptome Profiles in -Infected Gabonese Schoolchildren. Journal of Infectious Diseases, 2020. 222(12): p. 2103-2113.
27. Kaczorowski, K.J., et al., Continuous immunotypes describe human immune variation and predict diverse responses. Proceedings of the National Academy of Sciences of the United States of America, 2017. 114(30): p. E6097-E6106.
28. Yan, Z., et al., Aging and CMV discordance are associated with increased immune diversity between monozygotic twins. Immunity & Ageing, 2021. 18(1).
29. Carr, E.J., et al., The cellular composition of the human immune system is shaped by age and cohabitation. Nature Immunology, 2016. 17(4): p. 461-+.
30. Chakraborty, N.M., et al., Simplified Asset Indices to Measure Wealth and Equity in Health Programs: A Reliability and Validity Analysis Using Survey Data From 16 Countries. Global Health-Science and Practice, 2016. 4(1): p. 141-154.
31. Wikel, S.K., Modulation of the host immune system by ectoparasitic arthropods -Blood-feeding and tissue-dwelling arthropods manipulate host defenses to their advantage. Bioscience, 1999. 49(4): p. 311-320.
32. DHS. Wealth index 2016 [cited 2024 15.01.]; Available from: https://dhsprogram.com/topics/wealth-index/#:~:text=The%20wealth%20index%

20is%20a,water%20access%20and%20sanitation%20facilities.

33. Fisk, W.J., E.A. Eliseeva, and M.J. Mendell, Association of residential dampness and mold with respiratory tract infections and bronchitis: a meta-analysis. Environmental Health, 2010. 9.
34. TNBS. Tanzania population and housing census- Tanzania National Bureau of statistics(TNBS). 2022 [cited 2024; Available from:https://www.nbs.go.tz/index.php/en/census-surveys/population-and-housing-census.
35. TDHS-MIS, Tanzania Demographic and Health Survey and Malaria Indicator Survey (TDHS-MIS) 2015-16, in Ministry of Health, Community Development, Gender, Elderly and Children (MoHCDGEC) [Tanzania Mainland], Ministry of Health (MoH) [Zanzibar], National Bureau of Statistics (NBS), Office of the Chief Government Statistician (OCGS), and ICF. 2016. Tanzania Demographic and Health Survey and Malaria Indicator Survey (TDHS-MIS) 2015-16. Dar es Salaam, Tanzania, and Rockville, Maryland, USA: MoHCDGEC, MoH, NBS, OCGS, and ICF. 2016.
36. Maizels, R.M., Parasitic helminth infections and the control of human allergic and autoimmune disorders. Clinical Microbiology and Infection, 2016. 22(6): p. 481-486.
37. van Riet, E., et al., Cellular and humoral responses to influenza in Gabonese children living in rural and semi-urban areas. Journal of Infectious Diseases, 2007. 196(11): p. 1671-1678.
38. Djuardi, Y., et al., Immunological footprint: the development of a child's immune system in environments rich in microorganisms and parasites. Parasitology, 2011. 138(12): p. 1508-1518.
39. Mpairwe, H., R. Tweyongyere, and A.Elliott, Pregnancy and helminth infections. Parasite Immunology, 2014. 36(8): p. 328-337.
40. Poggensee, G., et al., A six-year follow-up of schoolchildren for urinary and intestinal schistosomiasis and soil-transmitted helminthiasis in Northern Tanzania. Acta Tropica, 2005. 93(2): p. 131-140.
41. McCall, L.I., et al., Home chemical and microbial transitions across urbanization. Nature Microbiology, 2020. 5(1): p. 108-115.
42. Vogt, M.B., et al., Mosquito saliva alone has profound effects on the human immune system. Plos Neglected Tropical Diseases, 2018. 12(5).
43. Libera, K., et al., Selected Livestock-Associated Zoonoses as a Growing Challenge for Public Health. Infectious Disease Reports, 2022. 14(1): p. 63-81.
44. Nutt, S.L., et al., The generation of antibody-secreting plasma cells. Nature Reviews Immunology, 2015. 15(3): p. 160-171.
45. Wrammert, J., et al., Rapid and Massive Virus-Specific Plasmablast Responses during Acute Dengue Virus Infection in Humans. Journal of Virology, 2012. 86(6): p. 2911-2918.
46. Fink, K., Origin and function of circulating plasmablasts during acute viral infections. Frontiers in Immunology, 2012. 3.

47. Vijay, R., et al., Infection-induced plasmablasts are a nutrient sink that impairs humoral immunity to malaria. Nature Immunology, 2020. 21(7): p. 790-+.
48. Hertz, J.T., et al., Chikungunya and Dengue Fever among Hospitalized Febrile Patients in Northern Tanzania. American Journal of Tropical Medicine and Hygiene, 2012. 86(1): p. 171-177.
49. Pankhurst, T.E., et al., MAIT cells activate dendritic cells to promote T(FH) cell differentiation and induce humoral immunity. Cell Rep, 2023. 42(4): p. 112310.
50. Jongo, S.A., et al., Safety, Immunogenicity, and Protective Efficacy against Controlled Human Malaria Infection of Sporozoite Vaccine in Tanzanian Adults. American Journal of Tropical Medicine and Hygiene, 2018. 99(2): p. 338-349.
51. Godfrey, D.I., et al., The biology and functional importance of MAIT cells. Nature Immunology, 2019. 20(9): p. 1110-1128.
52. Ben-Smith, A., et al., Differences between naive and memory T cell phenotype in Malawian and UK adolescents: a role for Cytomegalovirus? Bmc Infectious Diseases, 2008. 8.
53. Fourati, S., et al., Pan-vaccine analysis reveals innate immune endotypes predictive of antibody responses to vaccination. Nature Immunology, 2022. 23(12): p. 1777-+.
54. TDHS-MIS, The 2022 Tanzania Demographic and Health Survey and Malaria Indicator Survey (2022 TDHS-MIS). 202

Supplementary material

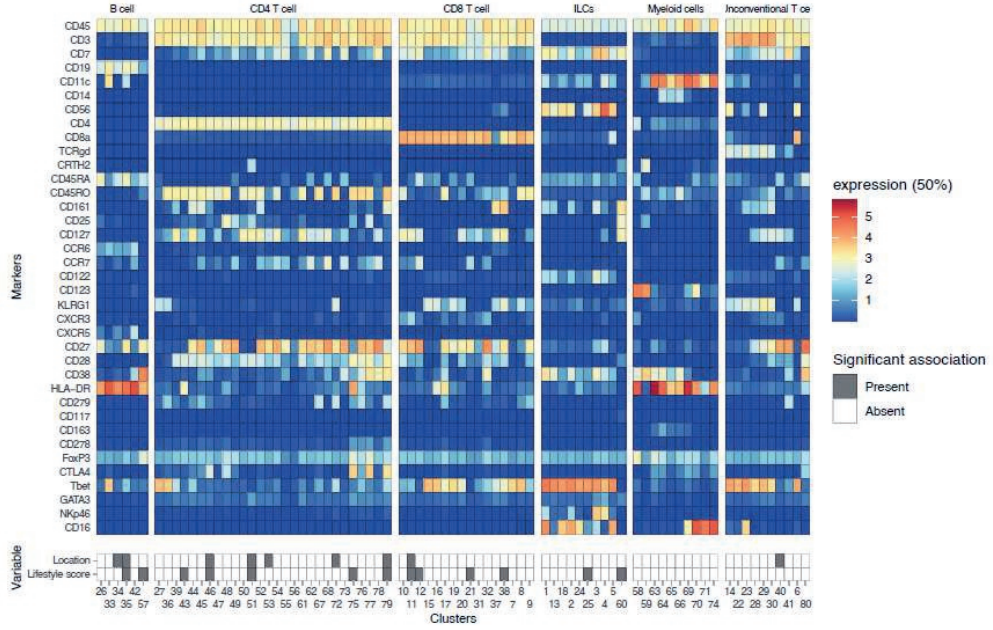

Figure S1 | Heatmap showing median marker expression for each cluster.

Clusters were based on SOM and hierarchical clustering. Each tile depicts the median expression of a given marker (rows) for a specific cluster (columns). The heatmap is stratified based on cell lineage. The bottom heatmap indicates which clusters were significantly associated with 1) location **(Figure 1)** and/or 2) lifestyle score **(Figure 3)**.

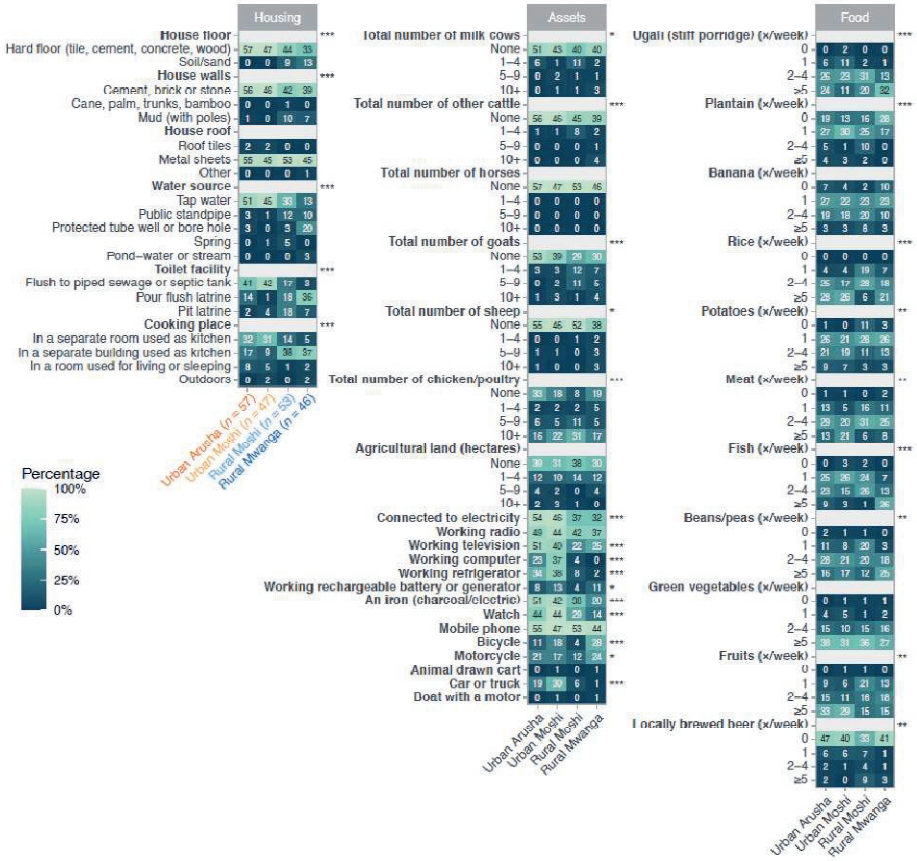

Figure S2 | Heatmap visualizing lifestyle questionnaire data.

N = 203 participants. Values represent the number of participants. Colours indicate the percentage of the total. Comparisons between locations were performed using Fisher's exact or chi-squared tests. Asterisks denote statistical significance (NS, non-significant; *, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.001$, $p \leq 0.0001$). See **Table S3**.

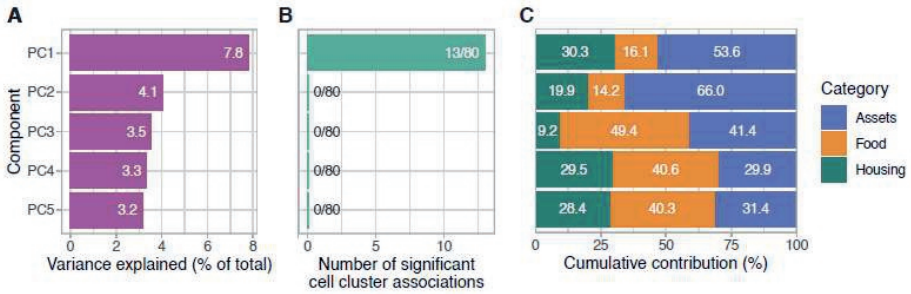

Figure S3 | MCA principal component variance explained, contributions and cluster associations.

A) Variance explained (% of total) for PC1-PC5. B) Number of significant cell cluster associations with PC1 (lifestyle score) to PC5 using modelling as described in the legend of **Figure 3**. C) Cumulative contributions (in percentage) of the variable categories by questionnaire data category (i.e. housing, assets and food, n = 38 questions and n = 118 variable categories) for PC1-PC5.

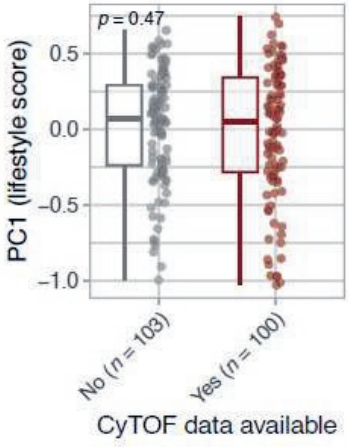

Figure S4 | Boxplots showing lifestyle score for individuals with and without mass cytometry immune profiles (n = 100). P-value determined using Student's t-test.

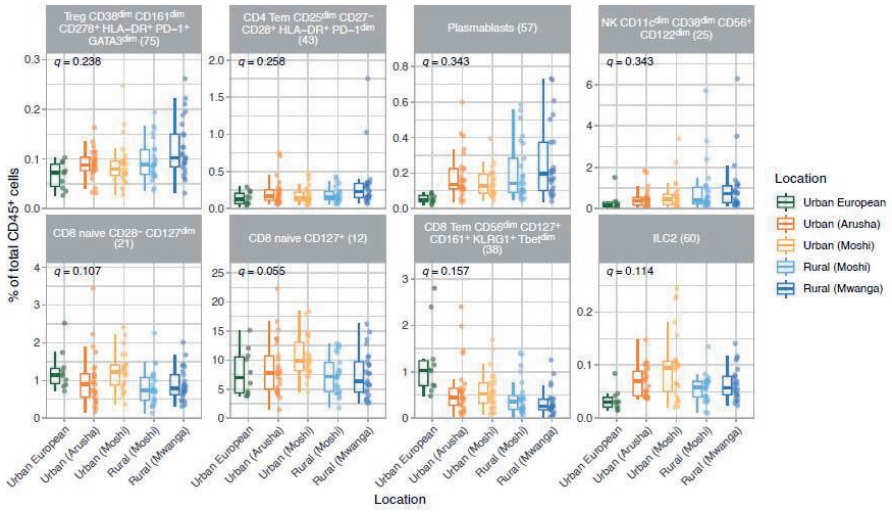

Figure S5 | Cell frequencies of clusters uniquely related to lifestyle score between locations.

Cell frequencies of clusters uniquely related to lifestyle score across rural and urban Tanzanian regions and urban Europeans **(Figure 3D)**. Boxplots represent the 25th and 75th percentiles (lower and upper boundaries of boxes, respectively), the median (middle horizontal line) and measurements that fall within 1.5 times the interquartile range (IQR; distance between 25th and 75th percentiles; whiskers). Significance of 'location' was assessed using analysis of variance (ANOVA)-tests comparing a simple (age [scaled] and sex [fixed effects] and sample ID [random effect]) and a full model (simple model with location as fixed effect added). P-values were corrected for multiple testing using the Benjamini-Hochberg method and referred to as q-values. Urban Europeans were included in the figure for visual comparisons and were not included in statistical tests.

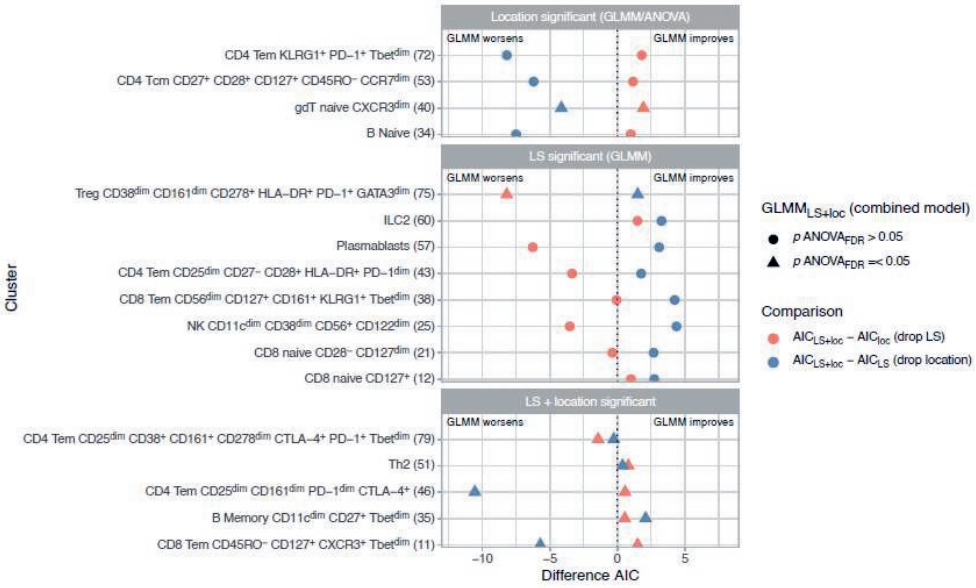

Figure S6 | Sensitivity analysis comparing location- and/or lifestyle-based models.

For each of the clusters that was significant in either location- and/or lifestyle-based models (n = 17), we additionally fitted a joint model, including both location and lifestyle (LS) (as well as age [scaled] and sex) as fixed effects and sample ID as random effect ($GLMM_{LS+loc}$). Statistical significance of the combined effect of location and lifestyle score was assessed by comparing $GLMM_{LS+loc}$ to an 'empty model' where both location and lifestyle score were removed using ANOVA (triangles indicate significant models). Akaike Information Criterion (AIC) (measure of model fit while accounting for model complexity) was compared between the 'combined model' (AIC_{LS+loc}) and the same model from which either lifestyle score (AIC_{loc}) or location (AIC_{LS}) was removed. Clusters were grouped according to the statistics shown in **Figure 1** and **Figure 3**, i.e. location significant, LS significant or LS + location significant clusters. Dropping location or lifestyle score from the combined model for location significant and LS significant clusters, respectively, worsened the combined model, indicating that location and lifestyle score were indeed related to distinct immune cell clusters. For most of the clusters in the LS + location significant group, dropping either location or lifestyle score did not change model performance, indicating that indeed here, location and lifestyle score may be more interrelated and capture similar information.

Table S1 | Baseline characteristics of the study population (*N* = 203).

Variable	Overall, N = 203	Urban Arusha, N = 57	Urban Moshi, N = 47	Rural Moshi, N = 46	Rural Mwanga, N = 53	p-value
Sex, female	100 (49%)	40 (70%)	26 (55%)	18 (39%)	16 (30%)	<0.001
Age	25.0 (22.0, 29.5)	25.0 (22.0, 30.0)	25.0 (23.0, 27.0)	26.0 (22.3, 31.0)	24.0 (21.0, 27.0)	0.165
Age categories						0.259
18-25	116 (57%)	30 (53%)	30 (64%)	22 (48%)	34 (64%)	
26-36	87 (43%)	27 (47%)	17 (36%)	24 (52%)	19 (36%)	
BMI	22.6 (20.5, 25.6)	22.2 (19.9, 25.8)	23.9 (22.2, 26.1)	22.4 (20.7, 25.0)	22.3 (20.3, 25.3)	0.183
Missing	1	1	0	0	0	
BMI classification						0.585
<18.5	13 (6.4%)	6 (11%)	3 (6.4%)	3 (6.5%)	1 (1.9%)	
18.5-24.9	130 (64%)	34 (61%)	27 (57%)	31 (67%)	38 (72%)	
25.0-29.9	39 (19%)	10 (18%)	11 (23%)	10 (22%)	8 (15%)	
>30	20 (9.9%)	6 (11%)	6 (13%)	2 (4.3%)	6 (11%)	
Missing	1	1	0	0	0	
Systolic blood pressure (mmHg)	120 (110, 128)	110 (109, 120)	110 (103, 120)	126 (118, 130)	122 (120, 130)	<0.001
Missing	1	1	0	0	0	
Diastolic blood pressure (mmHg)	73 (68, 80)	70 (67, 79)	70 (64, 78)	78 (72, 81)	76 (70, 80)	0.001
Missing	1	1	0	0	0	
Hemoglobin level g/dl	14.50 (13.35, 16.40)	13.90 (13.10, 15.00)	13.70 (12.30, 15.30)	15.25 (14.03, 16.58)	15.80 (14.00, 17.00)	<0.001
Random blood sugar, mmol-1^^	5.00 (4.50, 5.80)	4.80 (4.40, 5.50)	5.15 (4.53, 5.85)	5.50 (4.75, 6.20)	4.70 (3.90, 5.50)	0.002
Missing	1	0	1	0	0	
Highest level of education						<0.001
Primary	50 (25%)	4 (7.0%)	2 (4.3%)	27 (59%)	17 (32%)	
Secondary	74 (36%)	18 (32%)	11 (23%)	19 (41%)	26 (49%)	
College	40 (20%)	27 (47%)	6 (13%)	0 (0%)	7 (13%)	
University	39 (19%)	8 (14%)	28 (60%)	0 (0%)	3 (5.7%)	
Malaria	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	
Missing	1	0	1	0	0	
Helminth infection[a]	8 (3.9%)	0 (0%)	0 (0%)	6 (13%)	2 (3.8%)	0.002
Schistosomiasis[b]	7 (3.5%)	2 (3.6%)	1 (2.1%)	4 (8.9%)	0 (0%)	0.098
Missing	3	2	0	1	0	
Insurance status	51 (25%)	24 (42%)	23 (50%)	0 (0%)	4 (7.5%)	<0.001
Missing	1	0	1	0	0	
Occupation						<0.001
Farming	32 (16%)	2 (3.5%)	1 (2.1%)	23 (50%)	6 (11%)	
Elementary occupation	60 (30%)	14 (25%)	7 (15%)	13 (28%)	26 (49%)	
Student	47 (23%)	12 (21%)	23 (49%)	2 (4.3%)	10 (19%)	
Employed/ business owner	34 (17%)	15 (26%)	9 (19%)	4 (8.7%)	6 (11%)	
Not employed	30 (15%)	14 (25%)	7 (15%)	4 (8.7%)	5 (9.4%)	

N = 203 participants. Values represent number of participants (percentage of total) and median (interquartile range [IQR]) for categorical and continuous variables, respectively. Comparisons between locations were performed using Fisher's exact, chi-squared and Mann–Whitney U-test for categorical and continuous variables, respectively. [a] Stool was tested for helminths using the Kato-Katz method, testing for *Schistosoma haematobium*, *Schistosoma mansoni*, *Ascaris Lumbricoides*, hookworm and *Trichuris trichuria*. [b] Tested for schistosomiasis using the POC-CCA method, testing for *Schistosoma haematobium* and *Schistosoma mansoni*.

Table S2 | Overview of identified cell clusters.

See spreadsheets available in this link Download: Download spreadsheet (16KB) https://www.sciencedirect.com/science/article/pii/S2666354624001418.

Table S3 | Descriptives of lifestyle score variables.

Characteristic	Urban Arusha, N = 57	Urban Moshi, N = 47	Rural Moshi, N = 53	Rural Mwanga, N = 46	p-value
House floor					<0.001
Hard floor (tile, cement, concrete, wood)	57 (100%)	47 (100%)	44 (83%)	33 (72%)	
Earth/sand	0 (0%)	0 (0%)	9 (17%)	13 (28%)	
House walls					<0.001
Cement, brick or stone	56 (98%)	46 (100%)	42 (79%)	39 (85%)	
Cane, palm, trunks, bamboo	0 (0%)	0 (0%)	1 (1.9%)	0 (0%)	
Mud (with poles)	1 (1.8%)	0 (0%)	10 (19%)	7 (15%)	
Missing	0	1	0	0	
House roof					0.257
Roof tiles	2 (3.5%)	2 (4.3%)	0 (0%)	0 (0%)	
Metal sheets	55 (96%)	45 (96%)	53 (100%)	45 (98%)	
Other	0 (0%)	0 (0%)	0 (0%)	1 (2.2%)	
Water source					<0.001
Tap water	51 (89%)	45 (96%)	33 (62%)	13 (28%)	
Public standpipe	3 (5.3%)	1 (2.1%)	12 (23%)	10 (22%)	
Protected tube well or bore hole	3 (5.3%)	0 (0%)	3 (5.7%)	20 (43%)	
Spring	0 (0%)	1 (2.1%)	5 (9.4%)	0 (0%)	
Pond-water or stream	0 (0%)	0 (0%)	0 (0%)	3 (6.5%)	
Toilet facility					<0.001
Flush to piped sewage or septic tank	41 (72%)	42 (89%)	17 (32%)	3 (6.5%)	
Pour flush latrine	14 (25%)	1 (2.1%)	18 (34%)	36 (78%)	
Pit latrine	2 (3.5%)	4 (8.5%)	18 (34%)	7 (15%)	
Cooking place					<0.001
In a separate room used as kitchen	32 (56%)	31 (66%)	14 (26%)	5 (11%)	
In a separate building used as kitchen	17 (30%)	9 (19%)	38 (72%)	37 (80%)	
In a room used for living or sleeping	8 (14%)	5 (11%)	1 (1.9%)	2 (4.3%)	
Outdoors	0 (0%)	2 (4.3%)	0 (0%)	2 (4.3%)	
Total number of milk cows					0.012
None	51 (89%)	43 (91%)	40 (75%)	40 (87%)	
1-4	6 (11%)	1 (2.1%)	11 (21%)	2 (4.3%)	
5-9	0 (0%)	2 (4.3%)	1 (1.9%)	1 (2.2%)	
10+	0 (0%)	1 (2.1%)	1 (1.9%)	3 (6.5%)	
Total number of other cattle					<0.001
None	56 (98%)	46 (98%)	45 (85%)	39 (85%)	
1-4	1 (1.8%)	1 (2.1%)	8 (15%)	2 (4.3%)	
5-9	0 (0%)	0 (0%)	0 (0%)	1 (2.2%)	
10+	0 (0%)	0 (0%)	0 (0%)	4 (8.7%)	
Total number of horses					>0.999
None	57 (100%)	47 (100%)	53 (100%)	46 (100%)	
1-4	0 (0%)	0 (0%)	0 (0%)	0 (0%)	
5-9	0 (0%)	0 (0%)	0 (0%)	0 (0%)	
10+	0 (0%)	0 (0%)	0 (0%)	0 (0%)	
Total number of goats					<0.001
None	53 (93%)	39 (83%)	29 (55%)	30 (65%)	
1-4	3 (5.3%)	3 (6.4%)	12 (23%)	7 (15%)	
5-9	0 (0%)	2 (4.3%)	11 (21%)	5 (11%)	
10+	1 (1.8%)	3 (6.4%)	1 (1.9%)	4 (8.7%)	
Total number of sheep					0.031
None	55 (96%)	46 (98%)	52 (98%)	38 (83%)	
1-4	0 (0%)	0 (0%)	1 (1.9%)	2 (4.3%)	
5-9	1 (1.8%)	1 (2.1%)	0 (0%)	3 (6.5%)	

10+	1 (1.8%)	0 (0%)	0 (0%)	3 (6.5%)	
Total number of chicken/poultry					<0.001
None	33 (58%)	18 (38%)	8 (15%)	19 (41%)	
1-4	2 (3.5%)	2 (4.3%)	2 (3.8%)	5 (11%)	
5-9	6 (11%)	5 (11%)	11 (21%)	5 (11%)	
10+	16 (28%)	22 (47%)	31 (60%)	17 (37%)	
Missing	0	0	1	0	
Agricultural land (hectares)					0.439
None	39 (68%)	31 (67%)	38 (72%)	30 (65%)	
1-4	12 (21%)	10 (22%)	14 (26%)	12 (26%)	
5-9	4 (7.0%)	2 (4.3%)	0 (0%)	4 (8.7%)	
10+	2 (3.5%)	3 (6.5%)	1 (1.9%)	0 (0%)	
Missing	0	1	0	0	
Connected to electricity	54 (96%)	46 (98%)	37 (70%)	32 (70%)	<0.001
Missing	1	0	0	0	
Working radio	49 (86%)	44 (94%)	42 (79%)	37 (80%)	0.185
Working television	51 (89%)	40 (85%)	22 (42%)	25 (54%)	<0.001
Missing	0	0	1	0	
Working computer	23 (40%)	37 (79%)	4 (7.7%)	0 (0%)	<0.001
Missing	0	0	1	0	
Working refrigerator	34 (60%)	38 (81%)	8 (15%)	2 (4.3%)	<0.001
Working rechargeable battery or generator	8 (15%)	13 (28%)	4 (7.5%)	11 (24%)	0.035
Missing	2	0	0	1	
An iron (charcoal/electric)	51 (89%)	42 (93%)	38 (72%)	20 (43%)	<0.001
Missing	0	2	0	0	
Watch	44 (77%)	44 (98%)	29 (55%)	14 (30%)	<0.001
Missing	0	2	0	0	
Mobile phone	55 (96%)	47 (100%)	53 (100%)	44 (96%)	0.283
Bicycle	11 (19%)	18 (38%)	4 (7.7%)	28 (61%)	<0.001
Missing	0	0	1	0	
Motorcycle	21 (37%)	17 (37%)	12 (23%)	24 (52%)	0.026
Missing	0	1	0	0	
Animal drawn cart	0 (0%)	1 (2.2%)	0 (0%)	1 (2.2%)	0.353
Missing	1	1	0	0	
Car or truck	19 (33%)	30 (64%)	6 (11%)	1 (2.2%)	<0.001
Boat with a motor	0 (0%)	1 (2.2%)	0 (0%)	1 (2.2%)	0.353
Missing	0	1	1	1	
Ugali (stiff porridge) (×/week)					<0.001
0	0 (0%)	2 (4.3%)	0 (0%)	0 (0%)	
1	6 (11%)	11 (23%)	2 (3.8%)	1 (2.2%)	
2-4	26 (46%)	23 (49%)	31 (58%)	13 (28%)	
≥5	24 (43%)	11 (23%)	20 (38%)	32 (70%)	
Missing	1	0	0	0	
Plantain (×/week)					<0.001
0	19 (35%)	13 (28%)	16 (30%)	28 (62%)	
1	27 (49%)	30 (64%)	25 (47%)	17 (38%)	
2-4	5 (9.1%)	1 (2.1%)	10 (19%)	0 (0%)	
≥5	4 (7.3%)	3 (6.4%)	2 (3.8%)	0 (0%)	
Missing	2	0	0	1	
Banana (×/week)					0.152
0	7 (13%)	4 (8.5%)	2 (3.8%)	10 (22%)	
1	27 (48%)	22 (47%)	23 (43%)	23 (50%)	
2-4	19 (34%)	18 (38%)	20 (38%)	10 (22%)	
≥5	3 (5.4%)	3 (6.4%)	8 (15%)	3 (6.5%)	
Missing	1	0	0	0	
Rice (×/week)					<0.001
0	0 (0%)	0 (0%)	0 (0%)	0 (0%)	
1	4 (7.0%)	4 (8.5%)	19 (36%)	7 (15%)	
2-4	25 (44%)	17 (36%)	28 (53%)	18 (39%)	
≥5	28 (49%)	26 (55%)	6 (11%)	21 (46%)	

Potatoes (×/week)					0.005
0	1 (1.8%)	0 (0%)	11 (21%)	3 (6.7%)	
1	26 (46%)	21 (45%)	28 (53%)	26 (58%)	
2-4	21 (37%)	19 (40%)	11 (21%)	13 (29%)	
≥5	9 (16%)	7 (15%)	3 (5.7%)	3 (6.7%)	
Missing	0	0	0	1	
Meat (×/week)					0.008
0	1 (1.8%)	1 (2.1%)	0 (0%)	2 (4.3%)	
1	13 (23%)	5 (11%)	16 (30%)	11 (24%)	
2-4	29 (52%)	20 (43%)	31 (58%)	25 (54%)	
≥5	13 (23%)	21 (45%)	6 (11%)	8 (17%)	
Missing	1	0	0	0	
Fish (×/week)					<0.001
0	0 (0%)	3 (6.4%)	2 (3.8%)	0 (0%)	
1	25 (44%)	26 (55%)	24 (45%)	7 (15%)	
2-4	23 (40%)	15 (32%)	26 (49%)	13 (28%)	
≥5	9 (16%)	3 (6.4%)	1 (1.9%)	26 (57%)	
Beans/peas (×/week)					0.005
0	2 (3.5%)	1 (2.1%)	1 (1.9%)	0 (0%)	
1	11 (19%)	8 (17%)	20 (38%)	3 (6.5%)	
2-4	28 (49%)	21 (45%)	20 (38%)	18 (39%)	
≥5	16 (28%)	17 (36%)	12 (23%)	25 (54%)	
Green vegetables (×/week)					0.625
0	0 (0%)	1 (2.1%)	1 (1.9%)	1 (2.2%)	
1	4 (7.0%)	5 (11%)	1 (1.9%)	2 (4.3%)	
2-4	15 (26%)	10 (21%)	15 (28%)	16 (35%)	
≥5	38 (67%)	31 (66%)	36 (68%)	27 (59%)	
Fruits (×/week)					0.003
0	0 (0%)	1 (2.1%)	1 (1.9%)	0 (0%)	
1	9 (16%)	6 (13%)	21 (40%)	13 (28%)	
2-4	15 (26%)	11 (23%)	16 (30%)	18 (39%)	
≥5	33 (58%)	29 (62%)	15 (28%)	15 (33%)	
Locally brewed beer (×/week)					0.011
0	47 (82%)	40 (85%)	33 (62%)	41 (89%)	
1	6 (11%)	6 (13%)	7 (13%)	1 (2.2%)	
2-4	2 (3.5%)	1 (2.1%)	4 (7.5%)	1 (2.2%)	
≥5	2 (3.5%)	0 (0%)	9 (17%)	3 (6.5%)	

N = 203 participants. Values represent number of participants (percentage of total). Comparisons between locations were performed using Fisher's exact or chi-squared tests. All variables (*n* = 38 variables), after mode imputation, were used to construct the lifestyle score. See **Figure S2**.

Table S4 | Mass cytometry antibody panel.

Label	Specificity	Clone	Supplier[a]	Cat no	Lot no	End dilution	Working dilution
^{89}Y	CD45	HI30	Fluidigm	3089003B	2203476-08	200	100
^{115}In	CD278 (ICOS)	C398.4A	Biolegend	313502	22-02-2022 MK	100	50
^{141}Pr	CD196 (CCR6)	G034E3	Fluidigm	3141003A	2201583-11	100	50
^{142}Nd	CD19	HIB19	Biolegend	302202	24-06-2020	500	250
^{143}Nd	CD117 (c-Kit)	104D2	Biolegend	313223	28-01-2020	500	250
^{145}Nd	CD4	RPA-T4	Fluidigm	3145001B	2202012-07	500	250
^{146}Nd	CD8a	RPA-T8	Fluidigm	3146001B	2108701-11	500	250
^{147}Sm	CD183 (CXCR3)	G025H7	Biolegend	353733	03-01-2018	100	50
^{148}Nd	CD14	M5E2	Biolegend	301802	30-05-2022	200	100
^{149}Sm	CD25 (IL-2Ra)	2A3	Fluidigm	3149010B	2104640-07	500	250
^{150}Nd	CD185 (CXCR5)	J252D4	Biolegend	356902	10-09-2019	500	250
^{151}Eu	CD123	6H6	Fluidigm	3151001B	2112140-01	500	250
^{152}Sm	TCRγδ	11F2	Fluidigm	3152008B	2110581-20	200	100
^{153}Eu	CD7	CD7-6B7	Fluidigm	3153014B	0282010	200	100
^{154}Sm	CD163	GHI/61	Fluidigm	3154007B	3321818	100	50
^{155}Gd	CD45RA	HI100	Fluidigm	3155011B	0492003	200	100
^{156}Gd	CD294 (CRTH2)	BM16	Biolegend	350102	30-05-2022	100	50
^{158}Gd	CD122 (IL-2Rb)	TU27	Biolegend	339002	01-02-2022	500	250
^{159}Tb	CD197 (CCR7)	G043H7	Biolegend	353237	11-09-2020	200	100
^{161}Dy	KLRG1 (MAFA)	REA261	Miltenyi	130-126-458	01-02-2022	500	250
^{162}Dy	CD11c	Bu15	Fluidigm	3162005B	2111081-25	500	250
^{164}Dy	CD161	HP-3G10	Fluidigm	3164009B	2111083-25	200	100
^{165}Ho	CD127 (IL-7Ra)	AO19D5	Biolegend	351302	24-09-2020	500	250
^{167}Er	CD27	O323	Biolegend	302839	11-09-2019	500	250
^{168}Er	HLA-DR	L243	Biolegend	307651	01-02-2022	200	100
^{170}Er	CD3	UCHT1	Fluidigm	3170001B	169104	200	100
^{171}Yb	CD28	CD28.2	Biolegend	302902	01-02-2022	200	100
^{172}Yb	CD38	HIT2	Fluidigm	3172007B	2108738-17	200	100
^{173}Yb	CD45RO	UCHL1	Biolegend	304239	11-09-2019	200	100
^{174}Yb	CD335 (NKp46)	9E2	Biolegend	331902	22-12-2020	500	250
^{175}Lu	CD279 (PD-1)	EH 12.2H7	Fluidigm	3175008B	2104621-07	500	250
^{176}Yb	CD56	NCAM16.2	Fluidigm	3176008B	2202917-03	500	250
^{209}BI	CD16	3G8	Fluidigm	3209002B	2112429-15	200	100

[a]Fluidigm, South San Francisco, CA, USA; BioLegend, San Diego, CA, USA; Miltenyi Biotech, Bergisch Gladbach, Germany. CCR, CC chemokine receptor. CD, cluster of differentiation. CRTH2, prostaglandin D2 receptor 2. CXCR, CXC chemokine receptor. HLA-DR, human leukocyte antigen-D related. IL-2R, interleukin-2 receptor. IL2RB, Interleukin-2 receptor subunit beta, IL2Ra, Interleukin-2 receptor subunit alpha, ICOS, inducible T-cell COStimulator, IL-7Rα, interleukin-7 receptor alpha. KLRG1, killer cell lectin-like receptor subfamily G member 1. MAFA, mast cell function-associated antigen. c-Kit, receptor tyrosine kinase, PD-1, programmed cell death protein 1. TCR, T cell receptor.

Chapter 5

Differences in the myeloid compartment at baseline are associated with variation in antibody response to yellow fever vaccination in urban and rural Tanzanians

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*Contributed equally

To be submitted

Abstract

Introduction: Variation in vaccine response is observed between populations living in distinct geographical areas. This is thought to be associated with an altered immune state at baseline.

Aim: Here, we examine the association between the baseline innate immune characteristics and the response to yellow fever (YF) vaccination in healthy Dutch as well as urban and rural Tanzanian volunteers.

Methods: Response to YF vaccination was assessed by measuring plasma levels of IgG specific to non-structural protein 1 (NS1) of the YF virus at days 0 and 178 post-vaccination using ELISA. Using spectral flow cytometry, peripheral blood mononuclear cells were profiled with a panel directed at the innate immune system. PBMC were also analyzed after stimulation with the YF vaccine, ssRNA40 and Staphylococcus aureus enterotoxin B (SEB) for 24 hours.

Results: Post vaccination, a greater proportion of Dutch compared to Tanzanians had a high antibody response to NS1, but no difference was seen between urban and rural Tanzanians, in spite of the heterogeneity in the endpoint anti-NS1 IgG levels. When comparing the high and low responders within the Tanzanian cohort, high responders had an increased frequency of classical monocytes compared to low responders, both *ex vivo* and upon stimulation. Low responding Tanzanians were characterized by decreased frequency of cDC2s and IFN-alpha+ CD1c- CD141- DCs, whereas IL-10 production by cDC2s in unstimulated cultures was increased compared to high responding Tanzanians.

Conclusion: Differences in the frequency of subsets belonging to the myeloid compartment and their cytokine production associated with the antibody response to YF vaccination in Tanzanians. These data might help the understanding of the immunological mechanisms underlying vaccine response variation, necessary to develop interventions to overcome vaccine hyporesponsiveness.

Introduction

Vaccination against infectious diseases saves millions of lives each year [1]. However vaccines are not yet used to their full potential due to low and variable efficacy of some across populations and geographical areas [2-6]. Differences in vaccine efficacy are most clearly be observed for new vaccines evaluated in controlled human infection studies, such as the live *Plasmodium falciparum* sporozoite (PfSPZ) vaccine that demonstrated an protective efficacy of 92.3% in malaria-naïve subjects in the US, whereas in a malaria-endemic area in Tanzania it only protected 20% of the recipients [4, 5]. Moreover, reduced responses upon have been reported for well-established vaccines such as the yellow fever (YF), as Ugandans have been reported to have significantly lower neutralizing antibody titers upon vaccination in comparison to Swiss vaccinees [6].

An important determinant of the response to vaccination is the immune state at baseline, as altered immune baseline profiles have previously been associated with reduced vaccine responses [6-8]. A study among children from Kenya and Gabon vaccinated with the malaria vaccine RTS,S/AS01, showed that the monocyte-to-lymphocyte (ML) ratio at baseline is a good predictor for the efficacy of this vaccine, with reduced vaccine efficacy in those with high ML ratio [7]. This aligns with a study into YF response in Swiss and Ugandans, showing that increased baseline monocyte counts and the frequency of intermediate monocytes were associated with reduced antibody responses [6]. Moreover, a higher frequency of activated B and CD8+ T cells at baseline was found in Ugandans than in Swiss, which negatively correlated with neutralizing antibodies upon vaccination. Together, these results indicate that heightened immune activation at baseline, as observed in Ugandans, may drive the reduced response to YF vaccination [6]. More recent studies have focused on identifying a universal baseline signature that is predictive across several vaccines [8, 9]. A study combining pre-vaccination transcriptome data of multiple studies to predict the immune response across 13 different vaccines, including YF and malaria, identified three endotypes that are defined by multiple sets of genes, including pro-inflammatory and interferon-stimulated genes [8]. Comparison of the vaccine response among these endotypes, showed that among these young American individuals with limited pre-exposure to infections with the most pro-inflammatory endotype demonstrated highest vaccine responses [8]. However, how well these signatures hold up across populations with different environmental exposures remains an open question, as populations from LMICs have not been included in such studies.

Besides variation in the immune system between countries, differences in immune profiles within countries also have been observed [10, 11]. A study employing a multi-omics approach to study the immune profiles of rural and urban Senegal and urban Dutch subjects, revealed a continuous trajectory of immune remodeling along the rural-urban gradient [10]. The immune trajectory of rural individuals in this study was more pro-inflammatory compared to urban Senegalese and Dutch as rural individuals had increased frequency of CD11c+ B cells, monocytes producing TNF-alpha and IL-1β and T helper 1 cells (Th1). Moreover, comparing the cellular immune profiles of individuals from two rural and two urban populations in Northern Tanzania has shown that individuals with a rural lifestyle (low lifestyle score) had a more activated immune system as their immune profile was characterized by expansion of atypical B cells, T helper 2 cells, regulatory T cells as well as activated CD4+ T cells expressing CD38, HLA-DR and CTLA-4 [11]. Those with an urbanized lifestyle (high lifestyle score), however, showed a less activated immune state illustrated by higher frequencies of naïve CD8+ T cells [11].

Although heterogeneity in immune profiles within countries has been reported, the extent to which they affect the response to vaccines remains largely unexplored. The current work examines the differences of the immune system at baseline among rural and urban Tanzanians compared to Dutch and studies their association with the response to YF vaccine. Given the prominent role of the innate immune system in the initiation of the vaccine response and the observed associations between baseline inflammation and reduced vaccine response, the preliminary analysis presented here focuses on the innate immune compartment. Moreover, to assess the immune response to YF vaccine we use IgG specific to non-structural protein 1 of the YF virus (anti-NS1 IgG), an accurate continuous parameter [12], rather than the neutralizing antibody titer, which is often used in a dichotomous or semi-quantitative manner to confirm seroconversion. By studying the innate immune state at baseline among different populations and their relation to vaccine response, we will deepen our understanding of the immunological mechanisms that underly variation in vaccine responses, which is needed to develop interventions to overcome vaccine hyporesponsiveness.

Material and Methods

Study design

The current study is part of a prospective longitudinal cohort study (CapTan) in a healthy, 18-35-year-old Tanzanian population from rural and urban Moshi, Northern Tanzania. Volunteers were randomized into a vaccine group, receiving a single dose of yellow fever vaccine (YF-17D, Sanofi-Pasteur France) via intramuscular injection, and a control group who did not receive any intervention or placebo. Data and samples were collected at baseline and thereafter over a period of six months. The study took place from February to August 2023. The study protocol was approved by the Ethical Board of the Kilimanjaro Christian Medical University College (No. 2588) and by the Tanzania National Ethical Committee Board (NIMR/HQ/R.8a/Vol.IX/4089). The study was registered under The Pan African Clinical Trial Registry (PACTR) with trial number PACTR202405738173023. In addition, samples were collected from 15 Dutch participants who were not vaccinated with YF before. All individuals received the YF vaccine (YF-17D, Sanofi-Pasteur, France) via subcutaneous injection and were followed up for six months. This study took place from March to September 2023 in the Leiden University Medical Centre in Leiden. The study was approved by the Medical Ethical Committee Leiden The Hague Delft (NL70951.058.19) and is registered as clinical trial (ClinicalTrials.gov, NCT05901454).

Study population

Description of the study areas from which the Tanzanian participants were enrolled were published before [11]. In short, the study was conducted in rural and urban Moshi location in the Kilimanjaro region in northern Tanzania. The district of Moshi city (urban Moshi) is the administrative, commercial and educational center of the region and most inhabitants practice a Western lifestyle with generally good quality sanitation. Rural Moshi is an area north of urban Moshi, higher up the slopes of the Kilimanjaro and therefore has an elevation of 2,000-2,100 meters above sea level (compared to 700-950 meters in urban Moshi). Most inhabitants of rural Moshi are involved in farming activities.

Participant enrollment and data collection

Inhabitants of the two regions were informed about the study through community leaders, gatherings and leaflets and all eligible participants (age 18-35 years and permanent residence of the study location) were asked to enroll. Following informed consent, 233 participants were

voluntarily screened for exclusion criteria, including previous YF vaccination, comorbidities, HIV infection and use of medication possibly affecting the immune system; methods have previously been described in detail [11]. Based on the in- and exclusion criteria, 48 were excluded, therefore 185 participants were included and randomized over the vaccine and control group, resulting in 155 vaccinated individuals and 30 individuals that served as control group. Data were collected in REDCap, a cloud-based electronic data collection system, with a server hosted at the Kilimanjaro Clinical Research Institute (KCRI) in Tanzania.

Sample collection and processing

Before vaccination (Day 0), stool, urine and blood samples were collected from all included participants. Urine samples were used to test for pregnancy and determine infections with *Schistosoma* species using point-of-care test for the circulating cathodic antigen (POC-CCA). Kato-Katz was performed on stool samples to detect *Schistosoma* and soil-transmitted helminth eggs. From blood collected in sodium heparin tubes peripheral blood mononuclear cells (PBMCs) were isolated and cryopreserved as described previously [13]. Blood collected in EDTA tubes, were centrifuged upon arrival to the KCRI laboratory and plasma was collected and stored at -80°C within two hours. To assess the pre- and post-vaccination antibody response to YF vaccination, plasma was collected both at Day 0 and Day 178 post-vaccination. For the Dutch study EDTA plasma and PBMCs were collected and processed at similar timepoints and using identical protocols.

Enzyme-linked immunosorbent assay (ELISA)

To assess the YF virus antibody response, IgG specific to Yellow Fever Virus NS1 Protein was measured in plasma samples using enzyme-linked immunosorbent assay (ELISA). High-binding half area 96-well ELISA plates (Corning) were coated with 12.5 ng/well Yellow Fever Virus NS1 Protein (NAC-YFV-NS1-100, The Native Antigen Company) in 0.1 M sodium carbonate buffer (pH 9.6) overnight at 4°C. Plates were washed thrice with washing buffer (0.05% Tween-20 in PBS) and blocked for 2h with 5% skimmed milk in PBS containing 0.1% Tween-20. Plates were incubated with 25 µL/well of 4-step 1:2.5 serially diluted sera with a starting dilution of 1:250 for 2h at RT. After washing 5 times, NS1-specific IgG were measured using goat anti-human IgG-HRP (109-035-098, Jackson Immuno Research) at 1:5000 in 0.5% skimmed milk in PBS with 0.1% Tween-20 and 1-step TMB substrate solutions (34021,

ThermoFischer Scientific). Measured absorbance values were normalized using standard curve obtained from IgG (I5154, Sigma Aldrich) with the known concentration on each plate.

Innate baseline profiling

Sample selection for innate baseline profiling

From the 155 vaccinated participants, 50 individuals (25 urban and 25 rural) were randomly selected for innate baseline profiling. Samples were randomly selected after excluding samples with incomplete datasets due to loss-to-follow up (n=17), positive pregnancy test at day 178 (n=6) or insufficient PBMCs (n=6). From the Dutch cohort all participants were selected, except for one that had insufficient PBMCs, which resulted in the inclusion of n=14 Dutch individuals. In total 64 individuals were selected for innate immune profiling that were measured in three batches. Each of these batches were matched for geographical location, age and sex and included a reference control to allow batch correction after acquiring.

***Ex vivo* assays spectral flow cytometry**

Cells were thawed and washed in RPMI 1640 supplemented with 100 U/mL penicillin, 100 µg/mL streptomycin, 1 mM pyruvate, 2 mM L-glutamine, 2mM Mg2+, 50 U/ml benzonase, and 20% Heat-inactivated Fetal Calf Serum (hiFCS). Thereafter, cells were resuspended in IMDM supplemented with 100 U/mL penicillin, 100 µg/mL streptomycin, 1 mM pyruvate, 2 mM L-glutamine, and 10% hiFCS, and adjusted to a concentration of 5 x 10^6 cells/mL. Then, cells were plated as 0.5 x 10^6 cells per well in a 96-well V bottom plate. The cells were washed in phosphate-buffered saline (PBS), stained for viability with LIVE/DEAD™ Fixable Blue (Thermofisher) and Human TruStain FcX™ (Biolegend), washed again twice in FACS buffer (PBS supplemented with 0.5% BSA, Roche) and then stained with 50 µL of surface staining cocktail. The surface staining antibody cocktail, prepared in FACS buffer with Brilliant Stain Buffer Plus (BD Biosciences) and True-Stain Monocyte Blocker™ (Biolegend), was added to the cells and incubated for 30 min at RT. The list of antibodies can be found in **Table S1**. Cells were then washed twice in FACS buffer and afterward fixed with the eBioscience™ FoxP3 Transcription Factor Staining Buffer Set (ThermoFisher) for 30 minutes at 4 ˚ C. Subsequently, cells were washed twice with FACS buffer and resuspended in 120 µL of FACS buffer until acquisition. All centrifugation steps before fixation were performed at 450 g at RT and after fixation at 800 g at 4 °C.

***In vitro* stimulation assay spectral flow cytometry**

After thawing the cells as described above, 0.5 x 10^6 cells per well were plated in a 96-well U-bottom plate and rested for 1 hour at 37 ˚ C under 5% CO2. After resting, cells were stimulated with either YF vaccine (YF-17D, Sanofi Pasteur, Lot nr: X3E042V) at a concentration of 50.000 IU/mL (0.01 MOI) dissolved in 10% hiFCS/IMDM, 2 µg/mL ssRNA40 (Invitrogen), 0.2 µg/mL staphylococcal enterotoxin B (SEB; Sigma-Aldrich), or 10% hiFCS/IMDM for 23 h at 37 ˚ C under 5% CO_2. During the last four hours of stimulation, 10 µg/mL Brefeldin A (Sigma-Aldrich) was added.

After stimulation, cells were resuspended in PBS containing 2 mM EDTA and left on ice for 15 minutes, before transferring the cells to a 96-well V-bottom plate for staining. Thereafter, cells were washed in PBS, stained for viability with LIVE/DEAD™ Fixable Blue (Thermofisher) and Human TruStain FcX™ (Biolegend), washed again twice in FACS buffer (PBS supplemented with 0.5% BSA, Roche) and then stained with 50 µL of surface staining antibody cocktail. The surface staining antibody cocktail, prepared in FACS buffer with Brilliant Stain Buffer Plus (BD Biosciences) and True-Stain Monocyte Blocker™ (Biolegend), was added to the cells and incubated for 30 min at RT. The list of antibodies can be found in **Table S1**. Cells were then washed twice in FACS buffer and afterward fixed and permeabilized with the eBioscience™ FoxP3 Transcription Factor Staining Buffer Set (ThermoFisher) for 30 minutes at 4 ˚ C. Subsequently, cells were washes twice with the Permeabilization buffer from the eBioscience™ FoxP3 Transcription Factor Staining Buffer Set and then stained with intracellular cocktail. The intracellular cocktail, prepared in the Permeabilization buffer with Brilliant Stain Buffer Plus (BD Biosciences), True-Stain Monocyte BlockerTM (Biolegend) and Human TruStain FcX™ (BioLegend), was added to the cells and incubated overnight at 4 ˚ C. Next day, cells were washed twice with eBioscience™ Permeabilization buffer and were then resuspended in 120 µL of FACS buffer until acquisition. All centrifugation steps before fixation were performed at 450 g at RT and after fixation at 800 g at 4 °C.

Cell acquisition

Cells were acquired on a 5L-Cytek Aurora instrument at the Leiden University Medical Center Flow Cytometry Core Facility (https://www.lumc.nl/research/facilities/fcf/) with the SpectroFlo® v2.2.0.3 software (Cytek Biosciences). As controls, an unstained cell sample (pooled from all donors of the batch) and a single-stain sample for each antibody were used,

which were either 0.5 x 10^6 million PBMC or 50 µl UltraComp eBeads™ (Invitrogen). All reference controls underwent the same protocol as the fully stained samples, including washes, buffers used, and fixation and permeabilization steps.

Data analysis and statistical analysis

Transformation of spectral flow cytometry data

To obtain live single cells, manual gating of live, single CD45+ cells was performed using the OMIQ software (www.omiq.ai). Thereafter, cell subsets were identified based on the expression of surface markers and according to a pre-defined gating strategy (**Figure S1-S2**). Data that was clustered was then exported as FCS files. The *ex vivo* samples were batch corrected using CytoNorm (on default settings) and the compensated expression matrices arcsinh transformed, with a cofactor of 6000, before the data was merged into one dataset. After running CytoNorm, one cell had a batch corrected value of negative infinity. This value was changed to be the 1% percentile expression value for that fluorophore, across all cells for that individual. Post CytoNorm, and arcsinh transformation, any values above 10 or below -10 were clipped. The compensated expression matrices for the stimulated samples were also arcsinh transformed, with a cofactor of 6000. No batch correction was carried out for the culture samples.

Dimensionality reduction

For both the *ex vivo* merged datasets, a subsample of 100,000 cells was extracted randomly from the merged datasets and FItSNE ran on the arcsinh transformed batch corrected expression values of the *ex vivo* data subset, and the arcsinh transformed expression values of the culture data subset. For FItSNE, every marker associated with fluorophore was included as part of the feature space, except for CD45 and live/dead. For the principal component analysis, only the *ex vivo* dataset was used. In this analysis the frequency as percentage of total CD45+ cells of all the cell types manually gated were used. In addition, the 95th percentile expression of the activation markers were scaled to a mean of 0 and a standard deviation of 1 and thereafter included in the principal component analysis.

Cytokine gating

Cell type specific created to enable classification cells into cytokine positive and cytokine negative cells. For each of the fifteen gated innate cell types, a threshold was calculated to be:

$$percentile(x, 99) \ + \ (\ percentile(x, 99) \ \times 0.2\ )$$

Where x is the arcsinh transformed expression of a given cytokine in a given innate cell type in the unstimulated condition (upon culturing with only media). For the media only, SEB, ssRNA40 and YF-17D samples, cells were assigned as producers of a particular cytokine if they were above the threshold for their cell type.

Statistical analyses

All pairwise comparisons were carried out using Mann-Whitney U test with Benjamini-Hochberg correction. The number of comparisons to adjust for was based on the number of lineages (i.e. 5), not the number of gated subsets. For multiple comparisons of more than 2 groups, a Kruskal-Wallis test was used, with Dunn's test to carry out post-hoc pairwise comparisons between the groups. The FDR and alpha were set to be 5%. Differences in cytokine producing cells between high and low responders were only considered if the median percentage was greater than 0 across all individuals, and differences in activation marker expression were considered if the median of the 95th percentile expression, across all individuals, was greater than 1. PERMANOVA was performed using the adonis function of the vegan R package with 999 permutations. The input to the function was a Euclidean distance matrix of each individual's position across the first two principal components. All statistical analyses were carried out in R version 4.4.1. The packages used, and their versions, are as follows: rstatix 0.7.2, cytolib 2.16.0, kohonen 3.0.12, CytoNorm 2.0.2, igraph 2.2.1, ggpubr 0.6.0, matrixStats 1.4.1, stringr 1.5.1, flowCore 2.16.0 and vegan 2.6-8.

Results

Characteristics of the study population and antibody responses to YF-17D vaccination

To investigate how the innate immune cell compartment differs in individuals across and within different geographical areas and how it may impact vaccine response, volunteers were recruited from rural and urban areas of Tanzania and an urban area of the Netherlands. In total, 185 Tanzanians and 15 Dutch were recruited for the study, with 93 Tanzanians from urban and

92 from the rural area. All the Dutch and 155 of the Tanzanians (n=78 rural and n=77 urban) were vaccinated with the YF-17D vaccine, the remaining 30 Tanzanians served as a control (no vaccine) group. Dutch vaccinees were on average younger than Tanzanian vaccinees and more females were included in the Dutch compared to the Tanzanian cohort (**Table S2)**. The characteristics of the Tanzanians that received the vaccine and that served as control group did not differ (**Table S3**).

The vaccine immunogenicity was determined by measuring IgG specific to Yellow Fever non-structural protein 1, here referred to as anti-NS1 IgG, at baseline and day 178 post-vaccination (~6 months). Data for both these timepoints was available from 147 of the 155 vaccinated Tanzanians (n=70 rural and n=77 urban) and 12 of the 15 Dutch individuals **(Figure 1A).** The demographics of the individuals for which plasma from both timepoints were available did not differ from that of the total vaccinated subjects (**Table S4-5)**. To examine the innate immune profile at baseline, we profiled the PBMC of 50 individuals (n=25 rural and n=25 urban) that were randomly selected from the 155 vaccinated Tanzanians. From the Dutch cohort all participants with sufficient PBMCs were included, which resulted in innate baseline profiling of 14 Dutch individuals (**Figure 1A**). The age, sex and anti-NS1 IgG levels of the individuals selected for innate baseline profiling did not significantly differ from that of all vaccinated subjects included in the study (**Table S4-5**).

When comparing the anti-NS1 IgG levels between the cohorts, the median antibody levels did not significantly differ between the groups, but within the groups heterogeneity in the antibody response was observed (**Figure S3A**). Visualizing the antibody response at baseline and at Day 178 in all vaccinated individuals together on a histogram showed two noticeable peaks (**Figure S3B**). These peaks were modelled using a two-component Gaussian Mixture Model (GMM) (**Figure 1B**). All but three of the pre-vaccination values and all but one of the post-vaccination values of the control group (no vaccine) were positioned into this lower peak (**Figure S3C-D**).Therefore individuals whose post-vaccination anti-NS1 IgG levels clustered in this peak were designated as “low responders”, while individuals in the higher peak were designated as “high responders”. Using this categorization, in total 70 individuals were designated as high responders, whereas 89 individuals were low responders (**Figure 1B**). Examining the antibody response per group, showed that 83% (n=10) of Dutch individuals were in the high responders group, whereas 38% (n=29) of the urban Tanzanians and 44%

(n=31) of the rural Tanzanians were high responders (**Figure 1C**). The proportion of high responders was significantly greater in Dutch compared to all Tanzanians, whereas the proportion in urban and rural Tanzania was similar (**Table S6**).

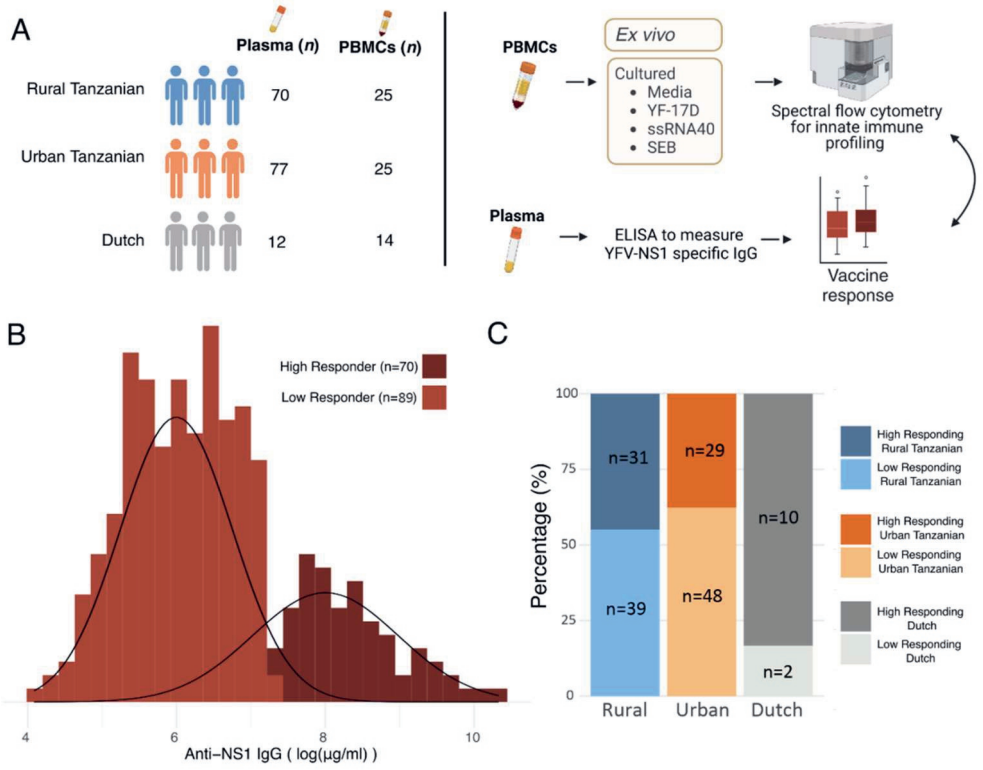

Figure 1. Studying the antibody response to yellow fever vaccination and innate immune baseline profile in rural and urban Tanzanians and Dutch vaccinees

(A) Graphical overview of the study population showing the number of individuals across the three geographical areas (Dutch and rural and urban Tanzanian) who had their PBMCs and plasma sampled (left). Graphical overview of the processing and acquisition steps for the PBMC and plasma samples (right). **(B)** Histogram visualising the anti-NS1 IgG levels of vaccinated individuals, with the individuals coloured by whether they were classified as high or low responders, based on a GMM model. The black lines show the distributions of the two Gaussian models. **(C)** Barplot showing the percentage of vaccinated individuals that belong to either high or low responders across the geographical areas, with each bar also showing the total number of individuals according to geographical area.

Baseline difference in the innate immune profiles of Dutch and Tanzanians

The innate immune profile was characterized before vaccination, at baseline, to assess whether signatures could be discerned that would predict antibody responses after vaccination. PBMCs were profiled from a random selection of 50 Tanzanians (18/32 high vs low responders equally

split between rural and urban, n=25 urban, n=25 rural) and 14 Dutch (10/2 high vs low responders, n=2 no day 178) vaccinees. One individual from the urban Tanzanians was removed due to low recovered cell count following staining. Spectral flow cytometry was performed using a panel to measure 26 markers for the *ex vivo* condition and 32 for the *in vitro* stimulated assay (**Table S1**). Manual gating using OMIQ, allowed us to identify monocytes, ILCs, Natural Killer (NK) cells, Myeloid Dendritic Cells (mDCs), plasmacytoid dendritic cells (pDCs) and Basophils (**Figure 2A**). Moreover, the monocyte population, here defined as CD3- CD19- CD88+ and/or CD14+, was further divided into four groups based on the expression of CD14 and CD16; classical monocytes (CD14+ CD16), intermediate monocytes (CD14+ CD16+), non-classical monocytes (CD14- CD16+) and CD14- CD16- monocytes. Moreover, the ILCs were classified as either ILC1, 2 and 3; the myeloid DCs could be subdivided into cDC1, cDC2 or CD1c-CD141- DC and the NK cells were split into three groups based on expression of CD16 and CD56 (**Figure S4**). To examine the activation status of these subsets, the 95th percentile expression of each activation marker (**Table S1**) for each of the manually gated innate cell types was determined.

To explore the overall innate immune baseline profile of rural and urban Tanzanians and Dutch individuals, a principal component analysis was performed using the frequencies of the manually gated innate cell types and the 95th percentile expression of activation markers in each of these cell types of the *ex vivo* samples. A significant separation (PERMANOVA p = 0.001) was seen across the first two principal components, with the Dutch being distinct from the rural and urban Tanzanians, whereas the rural and urban Tanzanians overlapped greatly **(Figure 2B**). To further examine the cell subsets and characteristics that contribute to the differences in the overall innate baseline profiles across the geographical areas, we compared the individual cell frequencies and expression of activation markers between rural and urban Tanzanians and Dutch (**Figure 2C-D**). With the exception of classical monocytes, which were higher in Dutch compared with Tanzanians, other myeloid cell subsets were significantly lower in the Dutch (**Figure 2C**). Moreover, the frequency of ILC2 and basophils, which indicate expansion of type-2 responses was significantly higher in urban Tanzanians compared to rural Tanzanians or the Dutch (**Figure 2C**). When comparing the activation status using the 95th percentile of the expression of the activation markers in the innate cell types a higher expression of TIM-3, an immunoinhibitory molecule, was observed in classical monocytes and cDC2s in Tanzanians compared to Dutch. Moreover, the expression of TIGIT, a marker of NK

activation and/or exhaustion, was significantly increased in CD16+ and CD16- NK cells of Tanzanians compared to Dutch equivalent cells (**Figure 2D**). Thus, the innate immune profile at baseline *ex vivo* of Dutch is distinct of that of Tanzanians, whereas urban and rural Tanzanian innate baseline profiles show great resemblance.

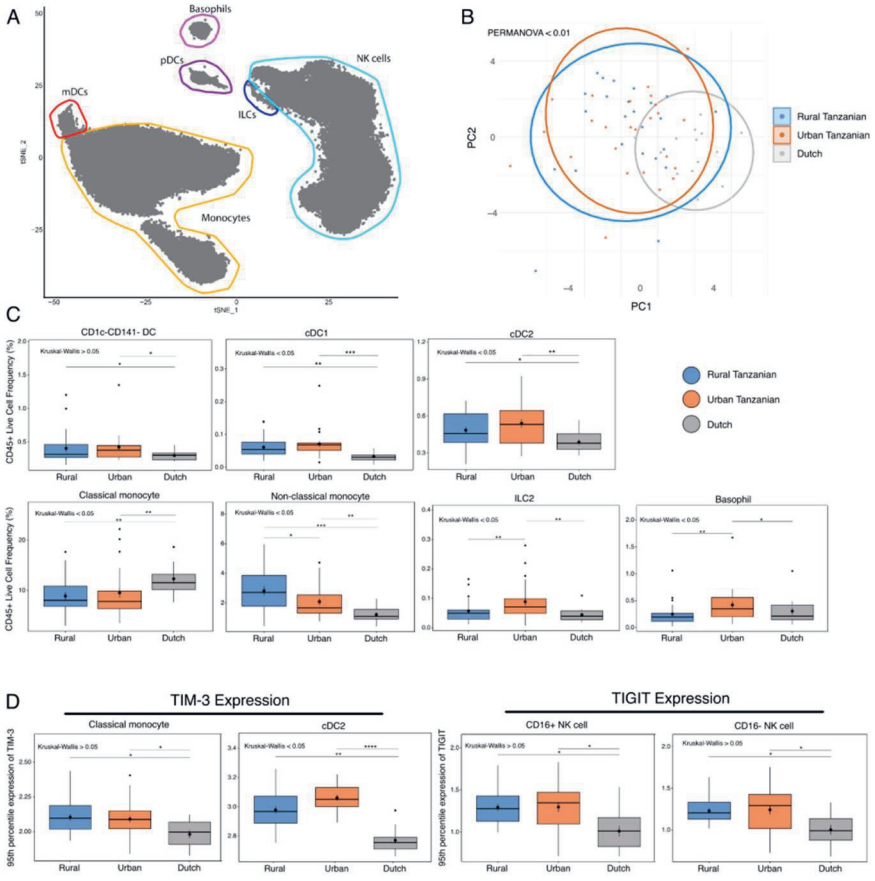

Figure 2. Innate immune profile at baseline of Dutch is distinct from that of Tanzanians

(A) FItSNE projection of a subset of the total innate cells from the *ex vivo* samples. Lineages of cells are outlined and annotated. **(B)** Principal component (PC) analysis of innate immune cells derived *ex vivo* from patients. PCs were generated from the 95th percentile expression of activation markers and frequency of innate cell types (as a percentage of total CD45+ cells). Individuals are coloured by the geographical area where the individual is from, and the coloured outline shows the 95th percentile area for each of the three geographical areas. PERMANOVA comparing the geographical areas is also shown. Boxplots showing the frequency of various innate immune cell types (as a percentage of total CD45+ cells) **(C)** and 95th percentile expression of activation markers TIGIT and TIM-3 **(D)** in rural Tanzanians, urban Tanzanians and Dutch. The boxplots are coloured by geographical area and contains the median (horizontal line), mean (dot) and 25th/75th percentiles with whiskers extending to ±1.5 × IQR. Differences in innate immune cell frequency and 95th percentile expression of activation marker across the three groups was assessed with Kruskal-Wallis test, with post-hoc pairwise comparisons achieved with Dunn's test, with Benjamini-Hochberg correction to account for multiple testing. ns = $p > 0.05$, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$, **** = $p < 0.0001$.

Frequency of *ex vivo* classical monocytes at baseline associates with post-vaccination antibody responses in Tanzanians

We next investigated whether differences between the vaccine responder groups, in terms of innate immune cells, could be detected at baseline. As only few Dutch were low responders and the innate immune signature at baseline of the Dutch was distinct from that of Tanzanians (**Figure 1C and 2B**), the high and low responders were compared for the Dutch and Tanzanian cohorts separately. Upon comparison, a significant increase in the frequency of classical monocytes was found in the high compared to low responding Tanzanians and this difference remained significant when Tanzanians were split between urban and rural (**Figure 3A-B**). As the frequency of classical monocytes in both high and low Dutch responders resembled that of high responding Tanzanians, no significant difference was found between high and low responding Dutch, although only few Dutch were low responders (**Figure 3C**). Besides differences in the frequency of classical monocytes, no statistically significant differences were found the frequency of other cell types or in the level of expression of activation markers within cell subsets between high and low responders.

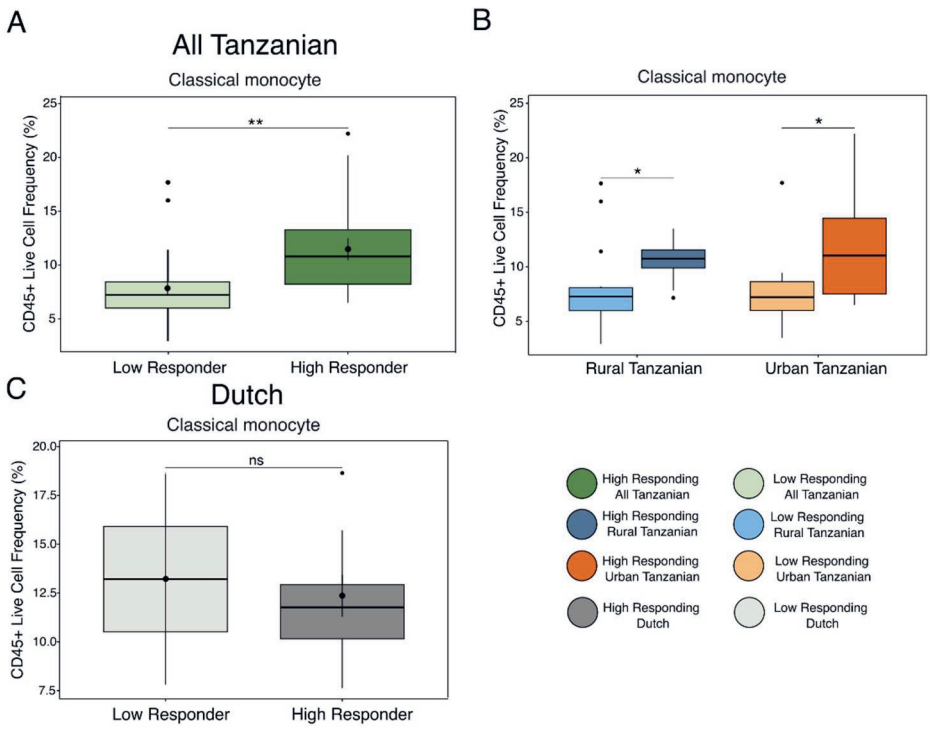

Figure 3. **High responding Tanzanians have increased frequency of *ex vivo* classical monocytes at baseline compared to responding Tanzanians:**
Boxplots showing the frequency of CD16-CD14+ monocytes (as a proportion of the total CD45+ cells) between the high and low responders of all Tanzanian **(A)**, rural and urban Tanzanians **(B) and Dutch individuals (C**). The boxplots are coloured by YF-17D vaccine response and geographical area, and contain the median (horizontal line) and 25th/75th percentiles with whiskers extending to ±1.5 × IQR. Changes in CD16-CD14+ monocyte frequency was assessed using a Mann-Whitney U test, with multiple test corrections with Benjamini-Hochberg. ns = $p > 0.05$, * = $p < 0.05$, ** = $p < 0.01$.

Cultured innate immune cell profiles associated with vaccine response in Tanzanians

We next examined whether responsiveness of innate immune cells to a challenge associates with antibody response to vaccination. To this end, *in vitro* response to either YF-17D, the TLR-8 ligand ssRNA40, the superantigen Staphylococcal Enterotoxin B (SEB) or medium as control (unstimulated culture) was determined. Similar to the *ex vivo* results, in the unstimulated culture, the frequency of classical monocytes were significantly increased in the high responding Tanzanians compared to the low responders (**Figure 4A**). In addition, upon culturing with medium the frequency of cDC2s and CD1c- CD141- DCs was significantly associated with the antibody response to YF in all Tanzanians. The frequency of cDC2s was increased in high compared to low responders, whereas for CD1c- CD141- DCs, the opposite was observed with lower frequencies in the high responding Tanzanians **(Figure 4A).** Interestingly, no such differences were seen in the high and low Dutch responders **(Figure S5).** In these unstimulated cultures, cDC2s producing IL-10 and CD1c- CD141- DCs producing IFN-alpha were different between high and low responding Tanzanians, as high responders had reduced IL-10+, but increased IFN-alpha+ CD1c- CD141- DCs **(Figure 4B).** Again, none of these differences between the high and low Dutch responders reached statistical significance (**Figure S5**).

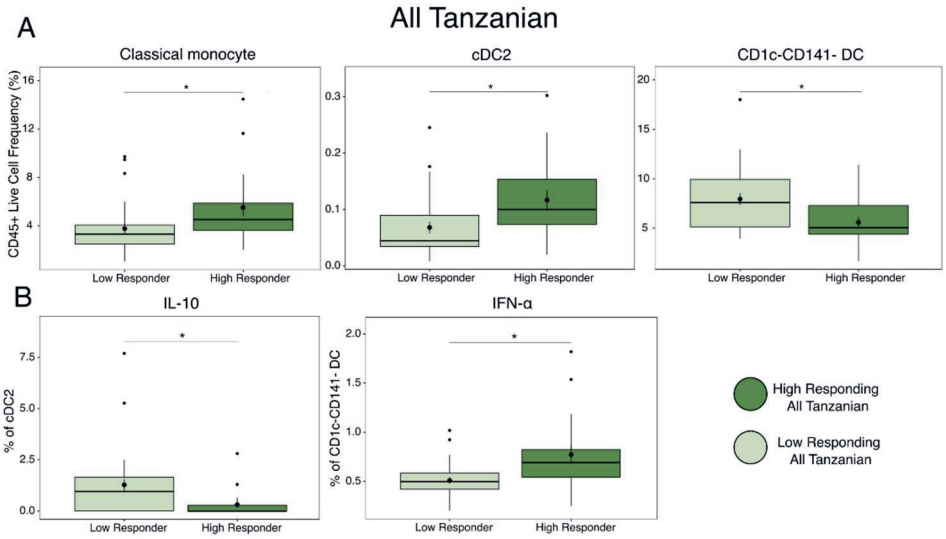

Figure 4. Frequency and cytokine production in dendritic cells in unstimulated culture are associated with the response to yellow fever vaccination in Tanzanians:

Boxplots showing the frequency of various innate immune cell types (as a percentage of total CD45+ cells) **(A)** and frequency of cytokine producing cells (as a percentage of the total parent cell) **(B)** between all Tanzanian high and low responders to the YF-17D vaccine, when the immune cells were cultured in media. The boxplots are coloured by YF-17D vaccine response and contains the median (horizontal line), mean (dot) and 25th/75th percentiles with whiskers extending to ±1.5 × IQR. Differences in innate immune cell frequency and cytokine producing cell frequency across between high and low responders was assessed with Mann-Whitney U test, with Benjamini-Hochberg correction to account for multiple testing. ns = $p > 0.05$, * = $p < 0.05$.

Upon stimulation with YF-17D, ssRNA40 and SEB, the innate cells responded by increased cytokine production, however when comparing the cytokine production between high and low responding Tanzanians we did not find statistical differences for any of the cytokines in any of the cell types after multiple testing correction (**Figure S6**). Comparison of the cell frequencies upon stimulation between high and low responders from Tanzania revealed that the differences largely reflected what was observed in unstimulated cultures (**Figure 4**). For the YF-17D stimulated culture, the frequency of CD1c-CD141- DCs was higher in the low responders, while that of classical monocytes and cDC2s was higher in the high responders, however these did not reach statistical significance (**Figure 5A**). The same trend, but with statistically significant differences was found in response to ssRNA40 with the frequencies of classical monocytes and cDC2s being higher but CD1c-CD141- DC lower in high responders compared

to low responders (**Figure 5B**). Finally, SEB stimulated cultures showed similar patterns (**Figure 5C**).

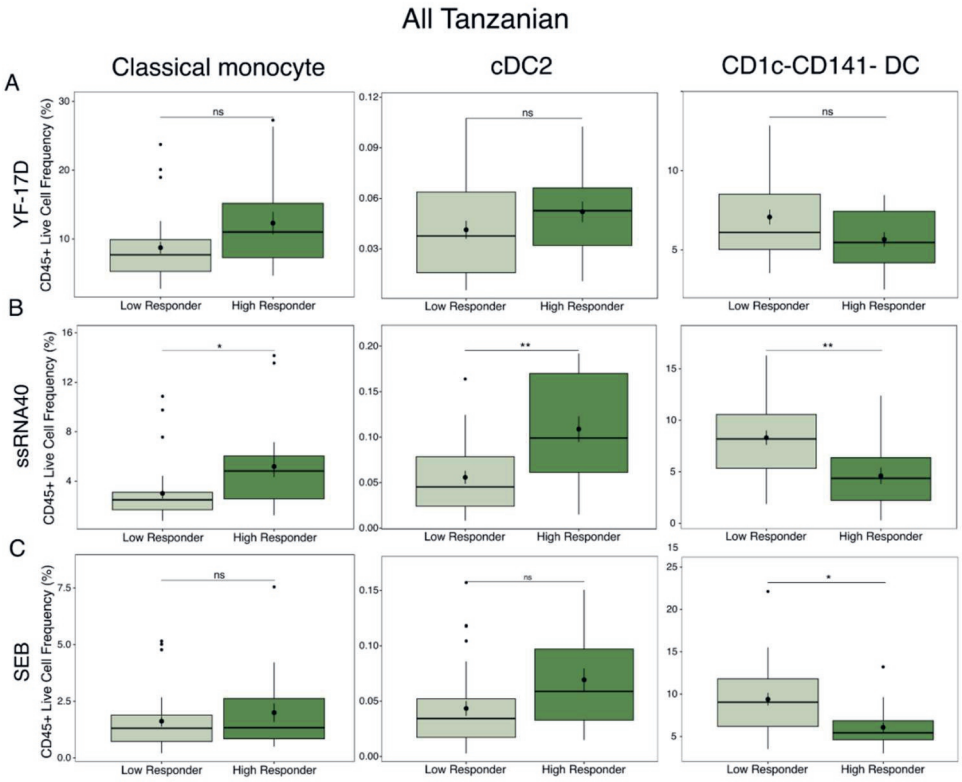

Figure 5. Changes in cell frequency upon culturing differ between stimuli, but cell types associated with antibody response to yellow fever vaccination show overlap across stimulations:
Boxplots showing the frequency of CD16-CD14+ monocytes (left), cDC2 (middle) and CD1c-CD141- DC (right) (as a proportion of the total CD45+ cells) between all Tanzanians who are high or low responders to the YF-17D vaccine. The boxplots are grouped based on whether the innate immune cell cultures were exposed to YF-17D **(A),** ssRNA40 **(B)** or SEB **(C).** The boxplots are coloured by YF-17D vaccine response and contains the median (horizontal line), mean (dot) and 25th/75th percentiles with whiskers extending to ±1.5 × IQR. Changes in the cell type frequency was assessed using a Mann-Whitney U test, with correction for multiple comparisons being achieved with Benjamini-Hochberg. ns = $p > 0.05$, * = $p < 0.05$, ** = $p < 0.01$.

Discussion

In summary, examining the antibody response to YF vaccination across populations revealed that most Dutch were high responders. Although high frequency of low responders were observed among Tanzanian vaccinees, no difference in the proportion of high and low responders between urban and rural Tanzania was observed. Comparing the innate baseline immune profiles across the geographical areas, showed that the overall innate immune profile at baseline of Dutch was distinct from that of Tanzanians and that Dutch had increased frequencies of classical monocytes and decreased frequencies of non-classical monocytes and multiple myeloid dendritic cell subsets. Within the Tanzanian cohort, high responders had a higher frequency of baseline classical monocytes, both *ex vivo* and upon *in vitro* stimulation, in comparison to low responders. When PBMCs were cultured, we found an increased frequency of cDC2s and reduced frequency of CD1c- CD141- DCs in low responding Tanzanians. Moreover, in unstimulated cultures both IL-10 production by cDC2s as well as IFN-alpha concentration in CD1c- CD141- DCs was increased in the high responders.

The increased antibody responses in Dutch compared to Tanzanian found in the current study, aligns with previous reports, as higher YF-17D specific CD8+ T cell and YF neutralizing antibody (nAb) responses have been found in Swiss compared to Ugandans [6]. As the antibody response between urban and rural Tanzanians did not significantly differ, this deviates from the findings of a recent study in Uganda. This study reported significantly higher nAb upon YF vaccination in urban individuals compared to helminth-endemic rural individuals 28 days post-vaccination and to malaria-endemic rural individuals 1 year after vaccination [14]. As helminth and malaria prevalence was low in the current study these discordant results may be explained by different levels of exposure to these pathogens between the Tanzanian and Ugandan cohorts. However, neither preventive treatment for malaria nor intensive praziquantel administration against *Schistosoma mansoni* improved the response to YF vaccine in the same study [15, 16]. As such, either past exposures or differences in the exposure to other environmental factors such as dietary habits should be considered [17].

To further understand the immunological mechanisms underlying variation in the response to YF vaccination, we profiled the innate immune system at baseline from a subset of Dutch and

Tanzanian vaccinees. The frequency of non-classical monocytes were decreased along the rural-urban gradient, while the frequency of classical monocytes showed the opposite trend. This aligns with findings of an earlier study showing increased proportions of intermediate and non-classical monocytes in Africans, while the frequency of classical monocytes followed a rural-urban gradient being highest in Europeans [18]. Within the Tanzanian cohort, the frequency of classical monocytes was significantly increased in high compared to low responders, both *ex vivo* and upon culturing, and when urban and rural were analyzed separately. The study of Muyanja *et al.* (2014) found a negative correlation between monocyte counts at baseline and YF nAb in the Ugandan cohort [6]. Moreover, when combining the data of Swiss and Ugandans vaccinees, the frequency of intermediate monocytes at baseline negatively correlated with YF nAb, although this was clearly driven by the distinct responses between geographical areas [6]. As characterization of the monocyte compartment can be challenging due to downregulation of markers upon culturing and may be affected by the gating strategy and cytometry panel used, the differential findings in our study and the study of Muyanja *et al*. (2014) should be interpreted with caution. A more standardized way of characterizing monocytes [19, 20] would allow comparison of different studies and will facilitate unraveling the role monocytes in response to (YF) vaccination.

Next to monocytes, differences in the proportion and cytokine response of myeloid dendritic cells between high and low responding Tanzanians were observed when PBMCs were cultured. In low responders, we detected an expansion of CD1c- CD141- DCs, a subset known to be enriched for type 1 interferon signaling and to share signatures with CD16+ monocytes [21]. Interestingly, although their frequency was decreased, the IFN-alpha production by these cells in unstimulated cultures was increased in high responders, indicating that in low responders the type 1 interferon production by these DCs might be hampered. Expansion of CD1c- CD141- DCS defective in their IFN signaling has previously been observed in SARS-CoV-2-infected individuals and these DCs were characterized by reduced ability for immune cross-talk and high mitochondrial activity [22]. Efforts should be made to further characterize the CD1c- CD141- DC population identified in the current study as this population is likely to comprise of multiple cell subsets given their high frequency upon culture. In addition to expansion of CD1c- CD141- DCs, increased frequency of IL-10+ cDC2s upon (unstimulated) culturing was observed in low versus high responding Tanzanians. A negative association between high IL-10 levels at baseline and reduced vaccine response has been reported

previously, as the level of IL-10 secreted by baseline PBMCs incubated with YF-17D overnight negatively correlated with YF nAb levels in Ugandans [6]. Given that IL-10 is an anti-inflammatory cytokine that regulates the T cell responses [23], enhanced IL-10 levels at baseline may suppress T cell responses upon vaccination, thereby hampering vaccine response. Indeed, a study in mice showed that increased levels of IL-10 were associated with reduced T cell responses and that blocking IL-10 during vaccination resulted in enhanced effector T cell responses and improved vaccine efficacy [24]. Together, these results indicate that alterations in subsets of myeloid dendritic cells are associated with reduced vaccine response. Therefore, single cell analysis of these dendritic cell subsets would be highly valuable to gain insight into the pathways involved and how these may be modulated to increase vaccine response.

Inherent to the preliminary nature of the analysis presented here, there are some limitations to be accounted for. In the current analysis, the characterization of the innate immune compartment was limited to identification of the main innate immune cell types by manual gating and the expression of the activation markers and cytokine production within these major innate immune subsets. Additional analysis such as sub-clustering of these major cell types which takes into account co-expression would enable us to identify and define smaller subsets and study their role in the response to YF vaccination. Moreover, the innate immune system of a limited number of subjects was profiled at baseline. As differences between the urban and rural populations included in the current study are likely to be relatively small, immune profiling of additional vaccinees from rural and urban Tanzanians might provide more power to detect differences.

In conclusion, upon YF vaccination the Dutch demonstrated increased antibody responses compared to Tanzanians, whereas the proportion of low responders was similar between urban and rural Tanzanians. Within the Tanzanian cohort, low responders were characterized by decreased frequency of classical monocytes and IFN-alpha production by CD1c- CD141- DCs, whereas IL-10 production by cDC2s in unstimulated cultures was increased. These results indicate that differences in the myeloid compartment at baseline may be associated with YF vaccine response, although additional characterization is needed to understand how specific cell subsets contribute to vaccine response variation. The insights obtained by this study contribute to our understanding of the immunological mechanisms underlying vaccine responses, which is essential for the development of interventions needed to overcome vaccine hyporesponsiveness.

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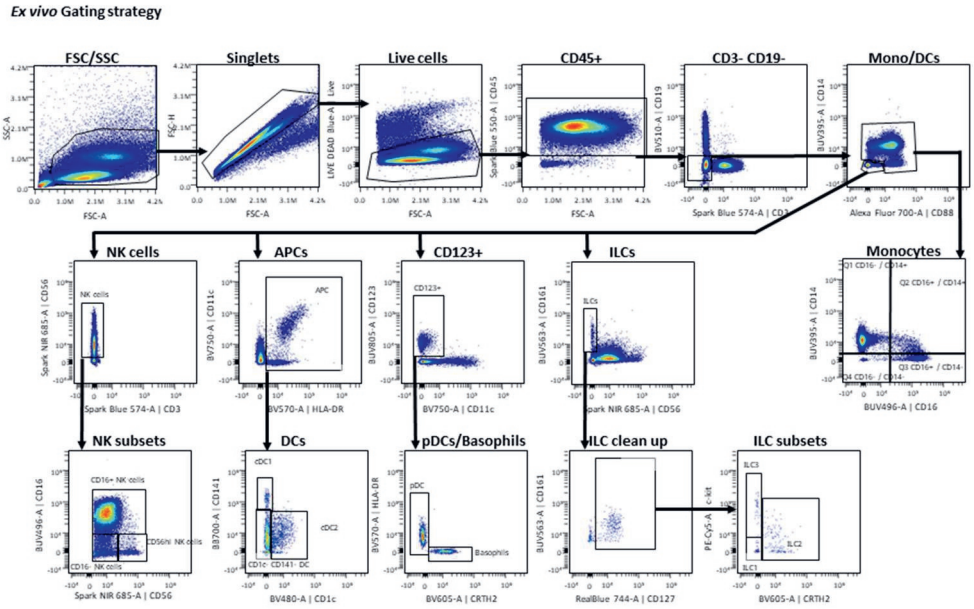
References

1. Li, X., et al., *Estimating the health impact of vaccination against ten pathogens in 98 low-income and middle-income countries from 2000 to 2030: a modelling study.* Lancet, 2021. **397**(10272): p. 398-408.
2. Abubakar, I., et al., *Systematic review and meta-analysis of the current evidence on the duration of protection by bacillus Calmette-Guerin vaccination against tuberculosis.* Health Technology Assessment, 2013. **17**(37).
3. Clarke, E. and U. Desselberger, *Correlates of protection against human rotavirus disease and the factors influencing protection in low-income settings.* Mucosal Immunology, 2015. **8**(1): p. 1-17.
4. Epstein, J.E., et al., *Protection against malaria by PfSPZ Vaccine.* Jci Insight, 2017. **2**(1).
5. Jongo, S.A., et al., *Safety, Immunogenicity, and Protective Efficacy against Controlled Human Malaria Infection of Sporozoite Vaccine in Tanzanian Adults.* American Journal of Tropical Medicine and Hygiene, 2018. **99**(2): p. 338-349.
6. Muyanja, E., et al., *Immune activation alters cellular and humoral responses to yellow fever 17D vaccine* Journal of Clinical Investigation, 2014. **124**(10): p. 4669-4669.
7. Warimwe, G.M., et al., *The Ratio of Monocytes to Lymphocytes in Peripheral Blood Correlates with Increased Susceptibility to Clinical Malaria in Kenyan Children.* Plos One, 2013. **8**(2).
8. Fourati, S., et al., *Pan-vaccine analysis reveals innate immune endotypes predictive of antibody responses to vaccination.* Nature Immunology, 2022. **23**(12).
9. Kotliarov, Y., et al., *Broad immune activation underlies shared set point signatures for vaccine responsiveness in healthy individuals and disease activity in patients with lupus.* Nature Medicine, 2020. **26**(4).
10. Manurung, M.D., et al., *Systems analysis unravels a common rural-urban gradient in immunological profile, function and metabolic dependencies.* Submitted for publication.
11. Pyuza, J.J., et al., *Lifestyle score is associated with cellular immune profiles in healthy Tanzanian adults.* Brain Behavior & Immunity-Health, 2024. **41**.
12. Liu, D.D., et al., *Preparation and application of yellow fever virus NS1 protein-specific monoclonal antibodies.* Journal of Medical Virology, 2021. **93**(6): p. 3374-3382.
13. de Ruiter, K., et al., *Helminth infections drive heterogeneity in human type 2 and regulatory cells.* Science Translational Medicine, 2020. **12**(524).
14. Natukunda, A., et al., *Schistosome and malaria exposure and urban-rural differences in vaccine responses in Uganda: a causal mediation analysis using data from three linked randomised controlled trials.* Lancet Global Health, 2024. **12**(11): p. e1860-e1870.
15. Nkurunungi, G., et al., *The effect of intensive praziquantel administration on vaccine-specific responses among schoolchildren in Ugandan schistosomiasis-endemic islands (POPVAC A): an open-label, randomised controlled trial.* Lancet Global Health, 2024. **12**(11): p. e1826-e1837.

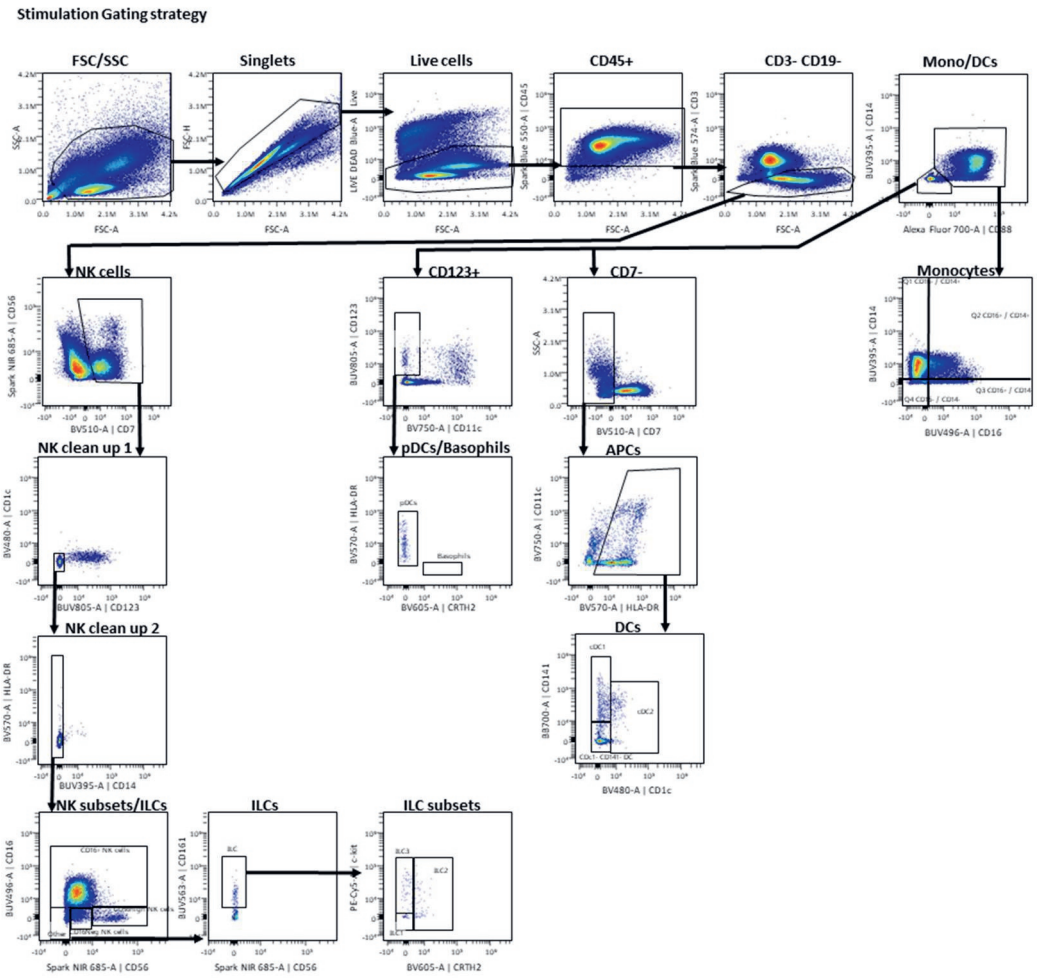
16. Zirimenya, L., et al., *The effect of intermittent preventive treatment for malaria with dihydroartemisinin-piperaquine on vaccine-specific responses among schoolchildren in rural Uganda (POPVAC B): adouble-blind, randomised controlled trial.* Lancet Global Health, 2024. **12**(11): p. e1838-e1848.
17. Temba, G.S., et al., *Urban living in healthy Tanzanians is associated with an inflammatory status driven by dietary and metabolic changes.* Nature Immunology, 2021. **22**(3).
18. Appleby, L.J., et al., *Sources of heterogeneity in human monocyte subsets.* Immunology Letters, 2013. **152**(1): p. 32-41.
19. Thomas, G.D., et al., *Human Blood Monocyte Subsets A New Gating Strategy Defined Using Cell Surface Markers Identified by Mass Cytometry.* Arteriosclerosis Thrombosis and Vascular Biology, 2017. **37**(8): p. 1548-+.
20. Tarfi, S., et al., *Technical, gating and interpretation recommendations for the partitioning of circulating monocyte subsets assessed by flow cytometry.* Cytometry B Clin Cytom, 2024. **106**(3): p. 203-215.
21. Villani, A.C., et al., *Single-cell RNA-seq reveals new types of human blood dendritic cells, monocytes, and progenitors.* Science, 2017. **356**(6335).
22. Liang, Q., et al., *A burns and COVID-19 shared stress responding gene network deciphers CD1C-CD141-DCs as the key cellular components in septic prognosis.* Cell Death Discovery, 2023. **9**(1).
23. Moore, K.W., et al., *Interleukin-10.* Annual Review of Immunology, 1993. **11**: p. 165-190.
24. Kelly, A.M., et al., *IL-10 inhibition during immunization improves vaccine-induced protection against Staphylococcus aureus infection.* Jci Insight, 2024. **9**(13).

Supplementary material



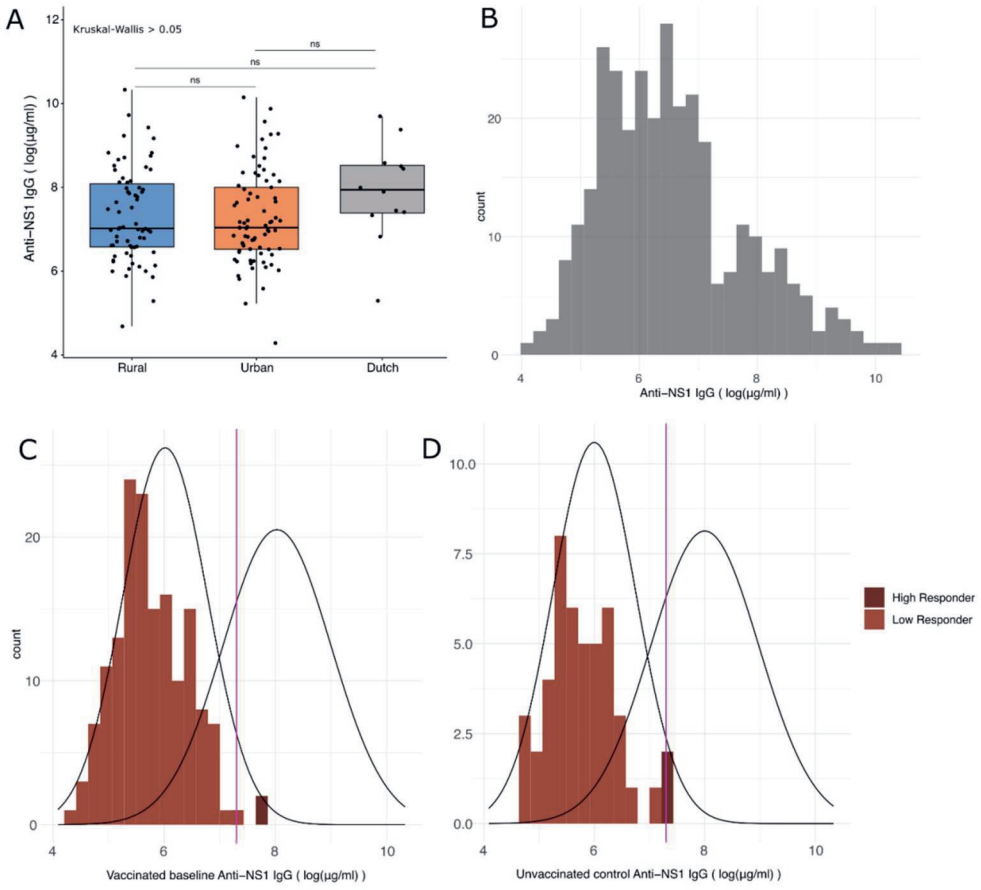
Supp. Figure 1 Gating strategy *ex vivo* spectral flow cytometry

Example of the gating strategies used to identify the cell types present in the *ex vivo* derived PBMCs as captured using spectral flow cytometry. The x and y axes represent the compensated fluorescence values of specific fluorophore/marker combinations or stain, with each dot representing an event and the events coloured by event density. Gating was carried out using OMIQ.



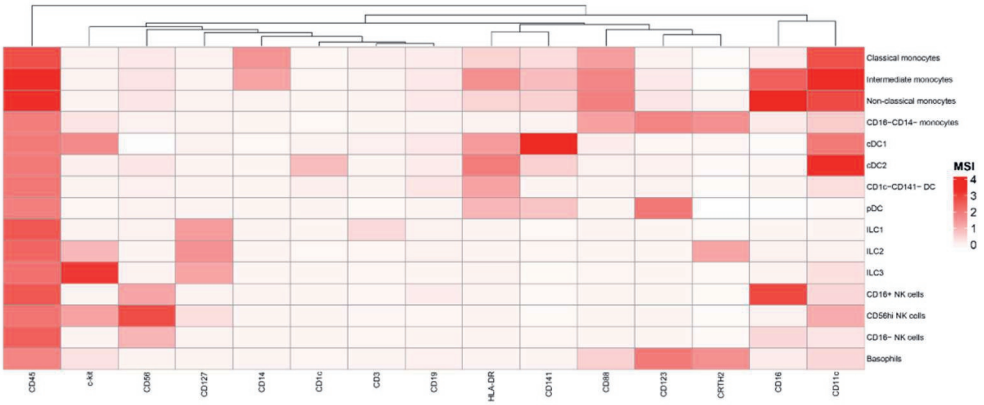
Supp. Figure 2 Gating strategy *in vitro* stimulation spectral flow cytometry

Example of the gating strategies used to identify the cell types present when PBMCs are cultured for 24 hours with either Yellow Fever vaccine, SSRNA40 or SEB as captured using spectral flow cytometry. The x and y axes represent the compensated fluorescence values of specific fluorophore/marker combinations or stain, with each dot representing an event and the events coloured by event density. Gating was carried out using OMIQ.



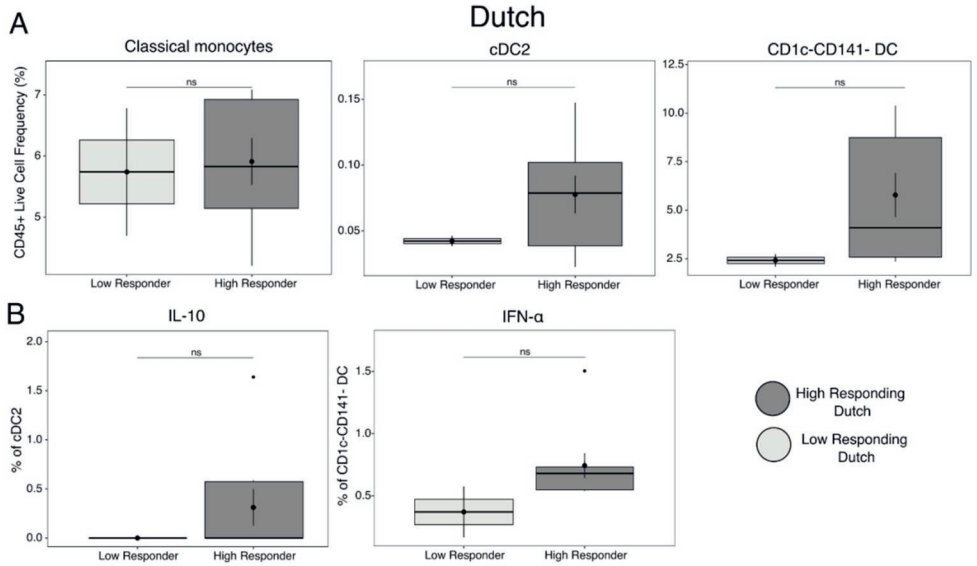
Supp. Figure 3 Yellow fever antibody response across geographical areas and in the control group

(A) Boxplot showing the Log_2 fold change of anti-NS1 IgG levels in individuals from baseline to 178 days post-vaccination with YF-17D. The boxplots are coloured by the geographical area that the individual is from and contains the median (horizontal line), mean (dot) and 25th/75th percentiles with whiskers extending to ±1.5 × IQR. Differences in log_2FC between the different areas was assessed with Kruskal-Wallis test, with post-hoc multiple comparisons achieved with Dunn's test using Benjamini-Hochberg correction. ns = $p > 0.05$. (B) Histogram visualising the anti-NS1 IgG levels from individuals at baseline and 178 days post-vaccination with YF-17D. Histogram visualising the anti-NS1 IgG levels of **vaccinated individuals at baseline (C)** and unvaccinated control individuals **(D)**, with the individuals coloured by whether they were classified as high or low responders, based on the GMM model shown in Figure 1B. The black lines show the distributions of the two Gaussian models while the purple vertical line denotes the decision boundary between the two clusters.



Supp. Figure 4 Expression of lineage markers in *Ex vivo* dataset cells

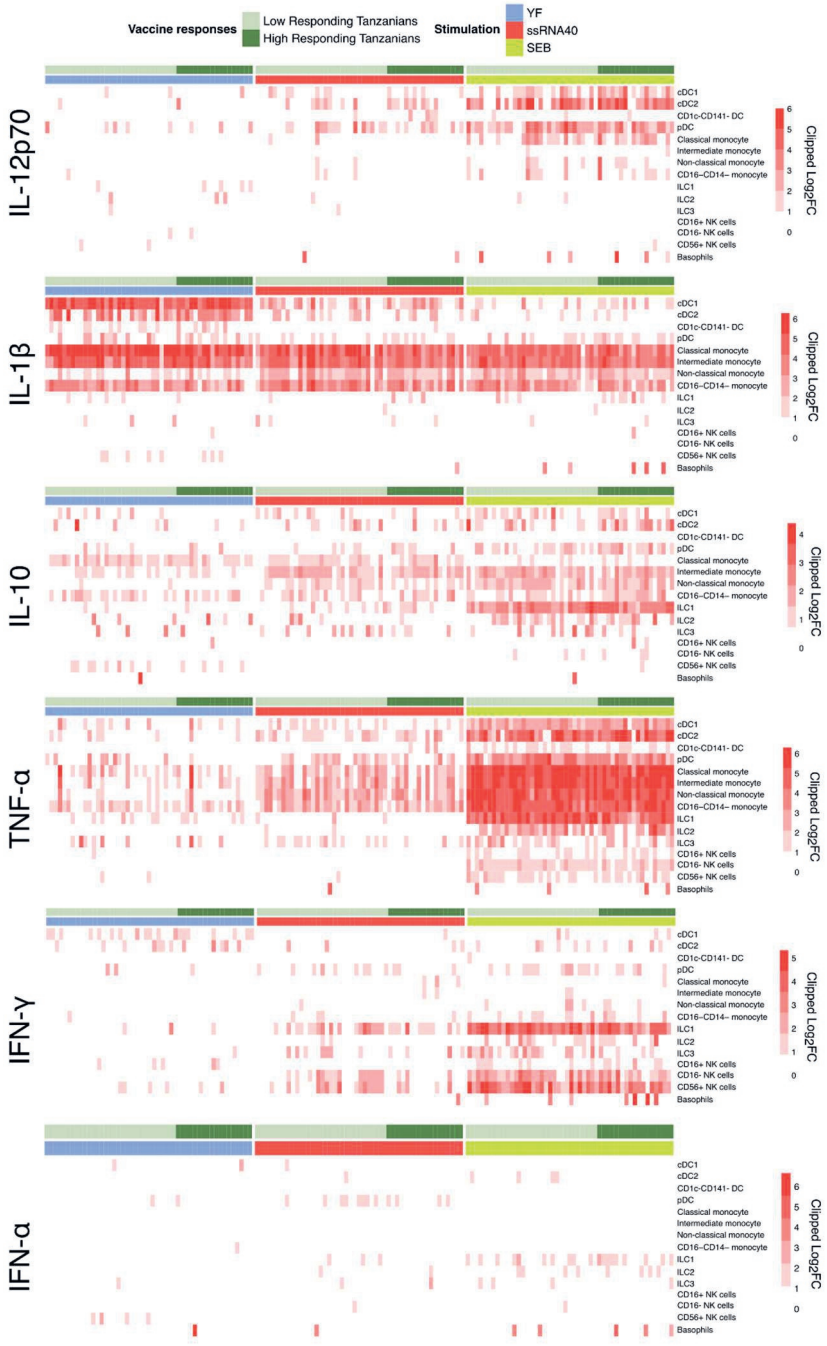
Heatmap showing the median signal intensity (MSI) of lineage markers across the OMIQ defined gates. Data shown is from all *ex vivo* spectral flow cytometry samples collected from Dutch and Tanzanian individuals.



Supp. Figure 5 Difference in frequency and cytokine production in medium condition between low and high responding Dutch vaccines

Boxplots showing the frequency of various innate immune cell types (as a percentage of total CD45+ cells) **(A)** and frequency of cytokine producing cells (as a percentage of the total parent cell) **(B)** between Dutch high and low responders to the YF-17D vaccine, when the immune cells were cultured in media. The boxplots are coloured by YF-17D vaccine response and contains the median (horizontal line), mean (dot) and 25th/75th percentiles with whiskers extending to ±1.5 × IQR. Differences in innate immune cell frequency and cytokine

producing cell frequency across between high and low responders was assessed with Mann-Whitney U test, with Benjamini-Hochberg correction to account for multiple testing. ns = $p > 0.05$.



Supp. Figure 6 Cytokine responses upon culturing with different stimuli across cell subsets
Heatmap showing the Log_2 fold change (Log_2FC) in cytokine+ cell frequency (as a percentage of total parent cell) between the media only culture and the YF-17D, ssRNA40 and SEB stimulated for all Tanzanian derived cultures. Each column represents an individual, with the individuals being grouped by whether they are a high or low responder (top grouping) and which stimuli was used during the culture (bottom grouping). A high Log_2FC indicates that the frequency of cytokine+ cells is higher in the given stimulated condition compared to the media-only. Log_2FC below 0 have been clipped to 0 for the purposes of this plot.

Table S1. Flow cytometry Antibody Panels

No.	Panel	Target	Fluorochrome	Dilution	Clone	Source	Catalogue	Type
1	Both	CD294 (CRTH2)	BV605	50	BM16	Biolegend	350121	Lineage
2	Both	CD279 (PD-1)	PE/Fire 700	50	A17188B	Biolegend	621621	Activation
3	Both	CD11c	BV750	50	B-ly6	BD	747459	Lineage
4	Both	CD159c (NKG2C)	BUV615	50	134591	BD	751059	Activation
5	Both	CD274 (PD-L1)	APC/Fire 810	50	MIH3	Biolegend	374515	Activation
6	Both	CD117 (c-kit)	PE/Cy5	50	104D2	Biolegend	313210	Activation
7	Both	CD178 (FasL)	PE/Cy7	50	NOK-1	Biolegend	306417	Activation
8	Both	CD161	BUV563	100	HP-3G10	BD	749223	Activation
9	Both	TIGIT	BV421	200	741182	BD	747844	Activation
10	Both	KLRG1	APC/Fire 750	200	SA231A2	Biolegend	367717	Activation
11	Both	CD14	BUV395	200	M5E2	BD	740286	Lineage
12	Both	CD45	Spark Blue 550	200	2D1	Biolegend	368549	Lineage
13	Both	CD88	AF700	200	S5/1	Biolegend	344314	Lineage
14	Both	CD366 (TIM-3)	BUV737	400	7D3	BD	568680	Activation
15	Both	CD123	BUV805	400	6H6	BD	751840	Lineage
16	Both	CD40	BV785	400	5C3	Biolegend	334339	Activation
17	Both	CD3	Spark Blue 574	400	UCHT1	Biolegend	300487	Lineage
18	Both	CD56	Spark NIR 685	800	5.1H11	Biolegend	362563	Lineage
19	Both	HLA-DR	BV570	800	L243	Biolegend	307637	Activation
20	Both	CD141	BB700	1600	1A4	BD	742245	Lineage
21	Both	CD16	BUV496	1600	3G8	BD	612944	Lineage
22	Both	CD163	BUV661	1600	MAC2-158	BD	752880	Activation
23	Both	CD1c	BV480	1600	F10/21A3	BD	746677	Lineage
24	Both	CD57	Pacific Blue	3200	HNK-1	Biolegend	359607	Activation
25	Ex vivo	CD127	RB744	50	HIL-7R-M21	BD	570607	Lineage
26	Ex vivo	CD19	BV510	400	HIB19	Biolegend	302241	Lineage
27	Culture	CD19	Spark Blue 574	100	SJ25C1	Biolegend	363048	Lineage
28	Culture	CD7	BV510	200	M-T701	BD	563650	Lineage
29	Culture	IFN-gamma	BV650	1600	4S.B3	Biolegend	502537	Cytokine
30	Culture	TNF-alpha	RealBlue780	12800	Mab11	BD	569091	Cytokine
31	Culture	IL12p70	APC	50	C11.5	BD	554576	Cytokine
32	Culture	IFN-alpha	PE-Vio 615	100	REA1013	BD	560097	Cytokine
33	Culture	IL-10	BV711	100	JES3-9D7	BD	564050	Cytokine
24	Culture	IL1b	FITC	1600	JK1B-1	Biolegend	508206	Cytokine

Ex vivo panel indicated that the antibody was only used in the panel to the *ex vivo* condition Culture panel indicates that the antibody was only used in the panel to measure after *in vitro* stimulation with medium, YF-17D, ssRNA40 or SEB for 24 hours Both panel indicates that the anybody was used for both in vitro stimulation and ex vivo measurements.

Table S2. Baseline characteristics of the vaccinated study population

Variable	N	Vaccinated Dutch N = 15[1]	Vaccinated Tanzanian N = 155[1]	p-value[2]
Sex	170			0.003
Female		13 (87%)	73 (47%)	
Male		2 (13%)	82 (53%)	
Age	170	20 (20, 22)	23 (21, 27)	0.004
Age Category	170			0.003
18-25		15 (100%)	100 (65%)	
26-35		0 (0%)	55 (35%)	

[1] n (%); Median (Q1, Q3)

[2] Pearson's Chi-squared test; Wilcoxon rank sum test; Fisher's exact test

N – 170 participants. Values represent the number of participants (percentage of total) and median (interquartile range [IQR]) for categorical and continuous variables respectively. Comparisons between vaccinated Dutch and all vaccinated Tanzanians were performed using Pearson's Chi-squared test for comparing Sex, Fisher's exact test for Age category and Wilcoxon rank sum test for Age.

Table S3. Baseline characteristics of the vaccinated and unvaccinated Tanzanian study population

Variable	N	Unvaccinated Tanzanian N = 30[1]	Vaccinated Tanzanian N = 155[1]	p-value[2]
Sex	185			0.3
Female		11 (37%)	73 (47%)	
Male		19 (63%)	82 (53%)	
Age	185	22 (20, 25)	23 (21, 27)	0.3
Age Category	185			0.2
18-25		23 (77%)	100 (65%)	
26-35		7 (23%)	55 (35%)	

[1] n (%); Median (Q1, Q3)

[2] Pearson's Chi-squared test; Wilcoxon rank sum test

N – 185 participants. Values represent the number of participants (percentage of total) and median (interquartile range [IQR]) for categorical and continuous variables respectively. Comparisons between vaccinated Dutch and all vaccinated Tanzanians were performed using Pearson's Chi-squared test for comparing Sex and Age category, and Wilcoxon rank sum test for Age.

Table S4. Comparisons of characteristics between all, plasma anti-NS1 IgG measured and PBMC sampled vaccinated Dutch individuals

Variable	Dutch, All N = 15[1]	Dutch, Plasma N = 12[1]	Dutch, PBMC N = 14[1]	p-value[2]
Sex				>0.9
Female	13 (87%)	11 (92%)	12 (86%)	
Male	2 (13%)	1 (8.3%)	2 (14%)	
Age	20 (20, 22)	21 (20, 22)	20 (20, 22)	>0.9
Anti-NS1, 178 days post-vaccination	7.94 (7.37, 8.54)	7.94 (7.37, 8.54)	7.99 (7.33, 8.58)	>0.9

[1] n (%); Median (Q1, Q3)

[2] Fisher's exact test; Kruskal-Wallis rank sum test

Values represent the number of participants (percentage of total) and median (interquartile range [IQR]) for categorical and continuous variables respectively. Comparisons between the three populations were performed using Fisher's exact test for comparing Sex and Kruskal-Wallis rank sum test for Age and anti-NS1 levels.

Table S5. Comparisons of characteristics between all, plasma anti-NS1 IgG measured and PBMC sampled vaccinated Tanzanian individuals

Variable	Tanzanian, All N = 155[1]	Tanzanian, Plasma N = 147[1]	Tanzanian, PBMC N = 50[1]	p-value[2]
Sex				>0.9
Female	73 (47%)	69 (47%)	24 (48%)	
Male	82 (53%)	78 (53%)	26 (52%)	
Age	23 (21, 27)	24 (21, 27)	22 (20, 26)	0.2
Anti-NS1, 178 days post-vaccination	7.03 (6.53, 8.04)	7.03 (6.53, 8.04)	6.90 (6.36, 8.15)	0.7

[1] n (%); Median (Q1, Q3)

[2] Pearson's Chi-squared test; Kruskal-Wallis rank sum test

Values represent the number of participants (percentage of total) and median (interquartile range [IQR]) for categorical and continuous variables respectively. Comparisons between the three populations were performed using Pearson's Chi-squared test for comparing Sex and Kruskal-Wallis rank sum test for Age and anti-NS1 levels.

Table S6. Differences in frequencies of vaccine responders between Dutch and Tanzanians, and Rural and Urban Tanzanians

Variable	N	Dutch, Plasma N = 12[1]	Tanzanian, Plasma N = 147[1]	p-value[2]	N	Rural Tanzanian, Plasma N = 70[1]	Urban Tanzanian, Plasma N = 77[1]	p-value[2]
Vaccine Responder	159			0.022	147			0.4
High Responder		9 (75%)	60 (41%)			31 (44%)	29 (38%)	
Low Responder		3 (25%)	87 (59%)			39 (56%)	48 (62%)	

[1] n (%)

[2] Pearson's Chi-squared test

N – 158 participants for Dutch and all Tanzanians, *N* – 146 for all Tanzanians. Values represent the number of participants (percentage of total). Comparisons between the frequency of high and low vaccine responders were performed using Pearson's Chi-squared test.

6

Chapter 6

Tanzanian gut microbiota profiles linked to high but rapidly waning yellow fever antibody titers

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Abstract

Vaccine responses vary across populations and are influenced by numerous intrinsic and extrinsic factors, including the gut microbiota. However, studies linking microbiota composition to vaccine immunogenicity in low- and middle-income countries are sparse. In this study, we examined the gut microbiota of 143 healthy rural and urban living Tanzanians who participated in a yellow fever vaccine (YF-17D) trial. We found significant differences in gut microbiota profiles between rural and urban participants. Rural-associated microbiota showed higher diversity and enrichment of taxa like *Prevotella* and Succinivibrio, which were linked to dietary intake patterns. Yellow fever neutralizing antibody titers were higher in rural compared to urban participants. Interestingly, a subset of urban individuals with a rural-like microbiota had higher antibody titers and faster waning than those with a more industrialized microbiota. These findings suggest that gut microbiota composition might be linked to vaccine immunogenicity, potentially outweighing the influence of living location.

Keywords: Yellow fever vaccine, YF-17D, microbiota, 16S-rRNA gene sequencing, neutralizing antibody, rural/urban differences, Tanzania.

Introduction

Vaccines play a critical role in the prevention of infectious diseases, especially in high-burden populations[1-5]. However, vaccine-induced immune responses vary across populations. Lower vaccine immunogenicity and efficacy ('hypo-responsiveness') is mainly observed in rural areas in low- and middle-income countries (LMICs), especially when compared to high-income countries (HICs) or to urban areas within the same countries[6-9]. Vaccine responses are impacted by a range of extrinsic and intrinsic factors, including host (age, sex, genetics, co-morbidities), behavioral (e.g. smoking) and environmental (rural/urban living location, season) factors, diet and nutrition, pre-existing immunity and vaccine factors (vaccine type, adjuvants, dose and administration route)[10-12]. Evidence suggests that the gut microbiome influences immune system development and regulation, thereby affecting vaccine responses[13-19]. Like vaccine responsiveness, the gut microbiota is highly variable across age, geographical locations and between people of different lifestyles[20-23]. Broad differences in gut microbiota composition and diversity have been observed between individuals living in rural and urban areas in LMICs[23-27]. Generally, these rural living individuals show a predominance of bacteria capable of polysaccharide degradation and fermentation, including *Prevotella* and other commensal bacteria such as the spirochaete *Treponema succifaciens*, which is enriched in non-industrialized populations[20,23,24,28,29]. In contrast, urban living individuals, who generally consume more processed foods and refined sugars, have a less diverse gut microbiota characterized by high abundance of *Bacteroides[23,26,30,31].*

Gut microbiota variation has been linked to both humoral and cellular vaccine responses[6] with the strongest associations found in mice receiving non-adjuvanted vaccines (e.g. non-adjuvanted influenza vaccine and inactivated polio vaccine)[32,33] The mechanisms underlying these associations include the production of immunomodulatory metabolites such as short-chain fatty acids[6,33]. In addition, it has been shown that microbial ligands from the microbiome may serve as natural vaccine adjuvants[34]. Indeed, studies in knockout mice have shown that innate sensing of

bacterial flagellin in the gut microbiome can provide adjuvant signals enhancing the antibody response to non-adjuvanted vaccines such as trivalent influenza vaccines (TIV) and polio vaccine[32]. The role of the microbiome in live-attenuated (self-adjuvanted) vaccine responses remains contentious. While studies on the yellow fever vaccine show that antibody responses in antibiotic-treated mice are similar to those in non-treated mice, suggesting minimal microbiome influence[32,34], there is also research indicating that the microbiome may impact responses to other live-attenuated vaccines, like BCG[35-37]. These findings so far suggest variation in associations between the host microbiome and the response to specific vaccines. In humans, similar in-human antibiotic intervention studies have not been performed for self-adjuvanted vaccines. Therefore, it is currently unknown whether the microbiota plays a role in potentiating the immune responses to self-adjuvanted vaccines, such as yellow fever vaccine and are generally considered prone to developing poor vaccine responses. Given that the gut microbiota can be modulated through diet and the administration of pre- or postbiotics, it may pose an interesting target for future strategies to enhance vaccine responses in these vulnerable populations.

We hypothesize that the gut microbiota profiles in rural and urban living Tanzanians are linked to vaccine responsiveness. To study this, we recruited healthy Tanzanian adults and vaccinated them with yellow fever vaccine (YF-17D). Stool and blood samples were collected over time. In line with previous work, we demonstrate that the gut microbiota composition is profoundly different between rural and urban living individuals and is linked to dietary habits. Variation in yellow fever neutralizing antibody responses may be at least partly explained by differences in microbiota community state types, potentially outweighing the impact of living location.

Results

Baseline characteristics of the study population

We enrolled 185 individuals living in rural and urban locations in Moshi, Northern Tanzania (**Figure 1a-b** and **Supplementary Figure 1**). Individuals were randomized into a vaccination group, including those who received the yellow fever vaccine (YF-

17D; $n = 155$) or a non-vaccinated control group ($n = 30$). All individuals were followed over six months and demographics and lifestyle variables (housing, assets and food history) were collected (**Supplementary Table 1** and **Supplementary Table 2**, **Supplementary Figure 2**). Plasma and/or stool samples were collected at baseline, day 28, 56 and 178 (**Figure 1c)**.

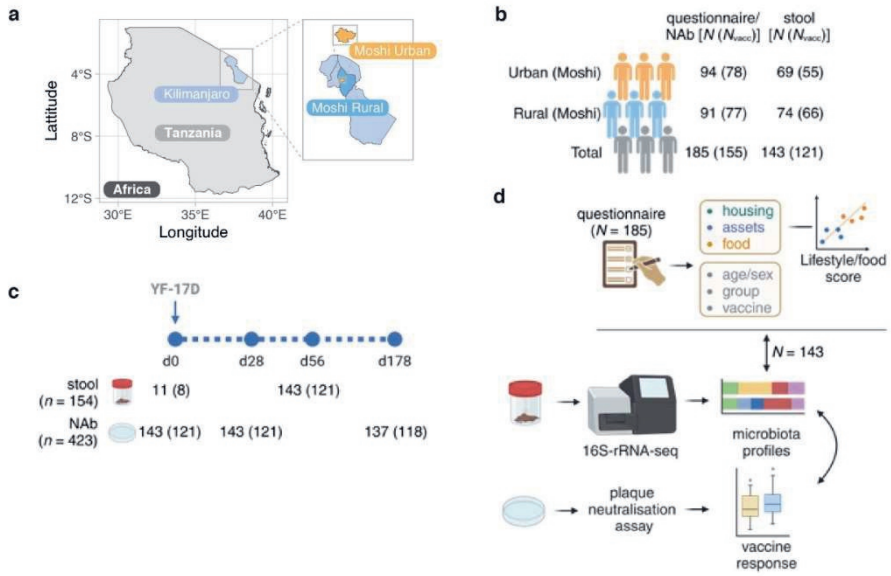

Figure 1 | Study overview.

a) Geographic map of study sites in Tanzania (Moshi Urban and Moshi Rural), within the Kilimanjaro region. b) Graphical representation of the number of urban and rural living participants included in the study and of those from whom stool samples for microbiota assays were available, stratified by the total number of participants (N) and the number of individuals who received the yellow fever (YF-17D) vaccine (N_{vacc}). c) Study design and number of samples at each time point. Numbers depicted as n (n_{vacc}), i.e. total number of samples (number of samples from vaccinated individuals). The number of samples in which neutralizing antibodies were measured only includes those samples with a matching stool sample. d) Graphical summary of study analyses. Questionnaire data from all included individuals (N = 185) were used in multiple correspondence analyses (MCA)/principal component analyses (PCA) to derive lifestyle/food scores. The associations between microbiota profiles and demographics and lifestyle factors (summarized in scores) and vaccine responses were assessed. Stool samples were available for microbiota sequencing from 143 of 185 (77.3%) individuals (n = 74 rural and 69 urban) (**Table 1**). A total of 154 stool samples were collected from 143 individuals (paired samples before and after vaccination were available from 11 individuals). The median age was 23.1 years (interquartile range [IQR], 21.2-27.1 years) and 43% were female (35% vs 52% in rural

and urban locations, respectively, $p = 0.040$). Baseline characteristics of the microbiota cohort were similar to the overall cohort (**Table 1** and **Supplementary Table 1**). The prevalence of parasitic infections was 4.9% and these infections were detected only in individuals from rural areas (**Table 1**).

Table 1 | Baseline characteristics of the study population (*N* = 143).

Variable	Overall, *N* = 143	Urban Moshi, *N* = 69	Rural Moshi, *N* = 74	p-value
Female sex	62 (43%)	36 (52%)	26 (35%)	0.040
Age	23.1 (21.2, 27.1)	23.0 (21.2, 24.6)	24.0 (21.2, 29.7)	0.209
Age categories				<0.001
18-25	94 (66%)	55 (80%)	39 (53%)	
26-36	49 (34%)	14 (20%)	35 (47%)	
BMI	22.5 (19.8, 25.1)	23.1 (20.4, 26.4)	22.2 (19.8, 24.4)	0.207
BMI classification				0.486
<18.5	16 (11%)	6 (8.7%)	10 (14%)	
18.5-24.9	91 (64%)	42 (61%)	49 (66%)	
25.0-29.9	27 (19%)	16 (23%)	11 (15%)	
>30	9 (6.3%)	5 (7.2%)	4 (5.4%)	
Systolic blood pressure (mmHg)	117 (106, 124)	110 (102, 120)	120 (110, 129)	0.003
Diastolic blood pressure (mmHg)	70 (65, 78)	70 (63, 76)	72 (67, 79)	0.099
Hemoglobin level g/dl	15.00 (13.60, 16.20)	14.30 (12.60, 15.60)	15.65 (14.20, 16.50)	<0.001
Random blood sugar, mmol-1^^	5.20 (4.80, 5.80)	5.30 (4.90, 6.00)	4.95 (4.70, 5.50)	0.008
Highest level of education				<0.001
Primary	43 (30%)	2 (2.9%)	41 (55%)	
Secondary	69 (48%)	38 (55%)	31 (42%)	
College	15 (10%)	14 (20%)	1 (1.4%)	
University	16 (11%)	15 (22%)	1 (1.4%)	
Helminth infection[a]	7 (4.9%)	0 (0%)	7 (9.5%)	0.014
Insurance status	63 (44%)	60 (87%)	3 (4.1%)	<0.001
Occupation				<0.001
Farming	28 (20%)	10 (14%)	18 (24%)	
Elementary occupation	50 (35%)	7 (10%)	43 (58%)	
Employed/business owner	58 (41%)	49 (71%)	9 (12%)	
Other	7 (4.9%)	3 (4.3%)	4 (5.4%)	
Received yellow fever vaccine	121 (85%)	55 (80%)	66 (89%)	0.116

N = 143 participants. Values represent number of participants (percentage of total) and median (interquartile range [IQR]) for categorical and continuous variables, respectively. Comparisons between locations were performed using Fisher's exact, chi-squared and Mann–Whitney U-test for categorical and continuous variables, respectively. [a] Stool was tested for helminths using the Kato-Katz method, testing for *Schistosoma mansoni*, *Ascaris lumbricoides*, hookworm and *Trichuris trichuria*. Additionally, urine was tested for *Schistosoma haematobium* and *Schistosoma mansoni* using the POC-CCA method.

Lifestyle and food scores vary with living location

Lifestyle questionnaire data were combined into a single lifestyle score to objectively gauge rural/urban living location-associated lifestyles ($N = 185$). To obtain the lifestyle score, we applied multiple correspondence analysis (MCA, a dimensionality reduction method for categorical data) to 38 questions (118 variable categories; **Supplementary Figure 3a)**. MCA separated individuals based on living location, especially across principal component (PC) 1 ('lifestyle score', **Supplementary Figure 3b)**, which captured 12.1% of the variation in questionnaire data. Rural individuals showed a larger spread across both PC1 and PC2, indicating they exhibit more diverse lifestyles. All variable categories contributed to the lifestyle score (**Supplementary Figure 3c)**, with variables related to possession of assets showing the highest cumulative contribution (57.8%). Variable categories most related to higher lifestyle score (associated with living in urban areas) included possession of household assets (e.g. working television, iron, watch, computer, refrigerator, radio, car/truck or computer). Variables related to housing quality, including the presence of a pit latrine, floors made of soil or sand and walls made of cane/palm/trunks/bamboo contributed to low lifestyle score (related to living in rural areas) (**Supplementary Figure 3d-e)**. PC2 scores were additionally driven by livestock-associated variables (**Supplementary Figure 3f-g**). Lifestyle scores (PC1) were similar for the overall ($N = 185$) and microbiota ($N = 143$) cohort (**Supplementary Figure 3h**).

Given the known association between diet and microbiome[38], we additionally developed a food score, based on 11 questions on frequency of consumption of specific food per week (ordinal variables). Using principal component analysis (PCA), variation in food consumption was captured across PC1 ('food score'), again showing a clear separation between rural and urban groups (**Supplementary Figure 4a-b)**. In contrast, PC2 covaried with the average reporting frequency across all food variables (**Supplementary Figure 4c)**, indicating interindividual differences in how the food questionnaire was filled out. Logistic regression analysis indicated that frequent consumption of fish, locally brewed beer (composed of fermented banana and millet) and green vegetables ($p \leq 0.009$) was significantly associated with rural living, whereas a carbohydrate-rich diet consisting of rice and potatoes was related to living in urban

areas (adjusted for all other food variables and sex, $p \leq 9.0 \times 10^{-4}$; **Supplementary Figure 4d)**. Both lifestyle and food score were included in microbiota association tests ($N = 143$; **Figure 1d)**.

Characteristics of microbiota data

To characterize the microbiota profiles of rural and urban living individuals, stool samples were subjected to Illumina MiSeq sequencing of V3-V4-region of the 16S-rRNA-gene, resulting in a median of 93,471 reads (range 58,482-124,868) per sample. A median of 68,648 reads (38,118-93,731) per sample remained after bioinformatic processing and quality filtering. We identified 3,354 amplicon sequence variants (ASVs) that occurred in ≥2 samples. ASVs could be aggregated into 272 genus-level taxa, 25 of which were part of the 'core microbiota' (genera detected in 80% of samples at ≥0.1% relative abundance). Highly abundant genera included *Prevotella* (mean relative abundance 27.6%), *Bacteroides* (7.9%), *Faecalibacterium* (7.7%), *Lachnospiraceae* (4.2%), *Oscillospiraceae* (3.5%), *Blautia* (2.6%) and *Succinivibrio* (2.4%). All microbiota analyses are conducted on samples collected at day 56, if not mentioned otherwise.

Microbiota profiles differ between rural and urban living Tanzanian adults

First, we investigated differences in microbiota profiles between rural and urban living Tanzanian adults. The number of observed ASVs was higher in rural compared to urban living Tanzanians (linear model, adjusted for vaccination status, age, sex and sequencing depth, $\beta = 89.0$, $p = 1.6 \times 10^{-8}$). Shannon diversity, a measure of within-sample microbial diversity, was higher in rural compared to urban living adults ($\beta = 0.368$, $p = 1.1 \times 10^{-6}$, respectively; **Figure 2a)**. Similarly, a higher number of observed genera was detected in rural compared to urban living individuals ($\beta = 13.2$, $p = 1.0 \times 10^{-4}$), yet genus-level Shannon diversity did not show differences between groups ($\beta = -0.019$, $p = 0.789$; **Figure 2b)**.

Next, we investigated the global differences in gut microbial community structure between rural and urban living Tanzanians. Principal component analysis (PCA on a centered-log-ratio [CLR]-transformed genus-level abundance matrix) indicated profound differences linked to rural/urban living location (**Figure 2c)**, which was confirmed by Permutational Multivariate Analysis of Variance (PERMANOVA; adjusting for vaccination, age and sex; R^2 = 9.0%, $p < 0.001$). Other important factors driving overall microbial community variation included lifestyle score (PC1 housing, assets and food-related questionnaire data), food score (PC1 food-related questionnaire data), highest level of education, PC2 (lifestyle) and sex (PERMANOVA, R^2 = 7.9%, 6.6%, 6.3%, 2.2% and 1.6%, respectively, $p \leq 0.005$), which are all at least partly collinear with rural/urban living location, which was therefore not considered in our models (**Table 1** and **Supplementary Table 1**, **Supplementary Figure 3b** and **Supplementary Figure 4b)**. No statistically significant association between helminth

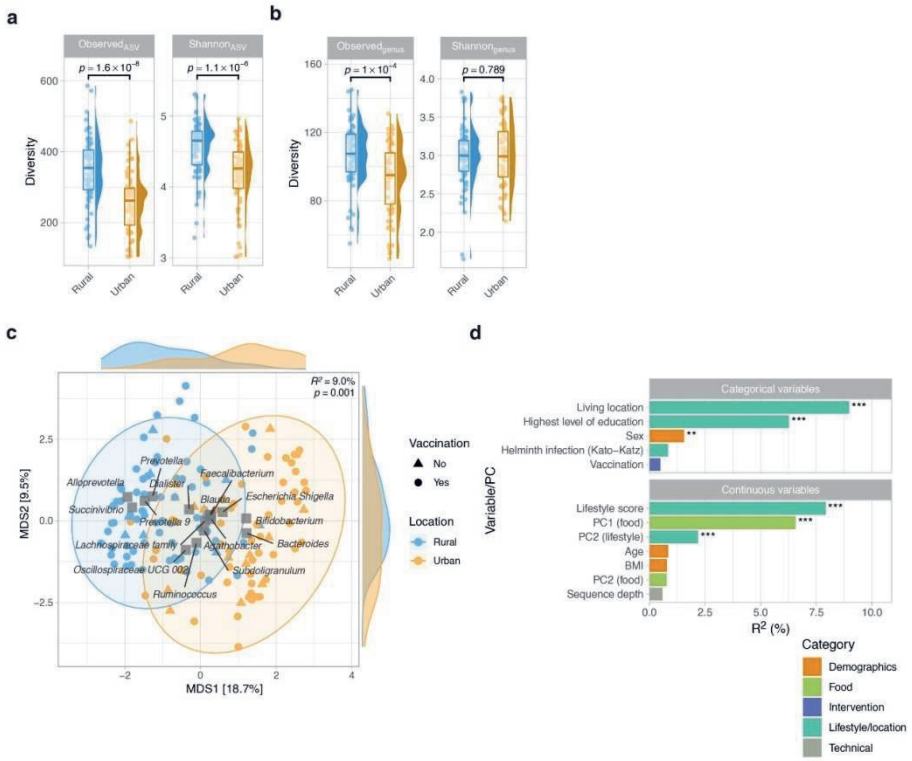

Figure 2 | Microbiota profiles differ across Tanzanians living in rural and urban regions.
a-b) ASV-level (a) and genus-level (b) Shannon diversity between study groups. Box plots represent the 25th and 75th percentiles (lower and upper boundaries of boxes, respectively), the median (middle horizontal line), and measurements that fall within 1.5 times the interquartile range (IQR; distance between 25th and 75th percentiles; whiskers). Density plots were used to visualize the distribution of data points. Statistical significance between groups was assessed using linear models with observed richness or Shannon diversity as outcome, adjusting for vaccination status, age, sex and sequencing depth. c) Principal component analysis (PCA) biplot using CLR-transformed genus-level microbiota features across day 56 samples. Percentages in square brackets denote the total variance explained by the first two principal coordinates. Each data point indicates a stool microbiota sample colored by group (rural/urban living). Ellipses reflect the data spread at a 95% confidence level. Density plots show the distribution of MDS1 (x-axis) and MDS2 (y-axis) score. The 15 highest ranking genera across all day 56 samples were simultaneously visualized (squares). R^2 and statistical significance of the association between group and the overall microbiota composition was assessed using PERMANOVA-test (1,000 permutations), while adjusting for vaccination status, age and sex. d) Bar plots indicating the effect size (R^2) of the association between demographic, technical, lifestyle or food-related variables (or derived scores based on these variables; see Supplementary Figure 3 and Supplementary Figure 4) and the overall microbiota composition. Statistical significance was assessed using PERMANOVA-tests. Each variable was tested separately. All analyses were adjusted for living location (except when assessing the impact of lifestyle/food-related variables, helminth infection status or sex), vaccination status, age and sex. Differential abundance analyses were primarily performed at genus level (MaAsLin2), testing all genera present at >0.1% abundance in ≥10% of samples. We found higher abundance of 34 genera in rural living individuals compared to 14 genera enriched in those living in an urban environment (109 genera tested; $p_{adj} < 0.05$ and $\log_2$-transformed fold change (FC) > 1.5; **Figure 3a)**.

infection status and microbiota composition was detected (R^2 = 0.8%, p = 0.184). Importantly, no association with vaccination status was detected (PERMANOVA, adjusting for living location, age and sex; R^2 = 0.5%, p = 0.787), suggesting that the vaccine had no impact on the gut microbiota composition at day 56 post-vaccination. In addition, no statistically significant effects for age, BMI or sequencing depth were observed (PERMANOVA, adjusting for living location, vaccination age and sex, as appropriate; R^2 = 0.8%, 0.8% and 0.6%, respectively, $p \geq 0.122$; **Figure 2d)**.

Specific taxa have previously been associated with industrialized and non-industrialized populations and are referred to as BloSSUM (bloom or selected in societies of urbanization/modernization) and VANISH (volatile and/or associated negatively with industrialized societies of humans) taxa respectively[22]. We found that urban-associated genera (15/109 genera tested) were significantly enriched for

BloSSUM taxa (9/14 urban-associated genera, compared to 6/95 non-urban-associated genera, Fisher's Exact test $p = 1.8\times10^{-6}$; **Figure 3b** and **2c)**, whereas rural living individuals were specifically devoid of these genera (1/34 compared to 14/75, $p = 0.034$; **Figure 3d)**. In contrast, genera associated with rural living location were not significantly enriched for VANISH taxa (total 18/109; 8/34 rural-associated genera, compared to 10/75 non-rural-associated genera, Fisher's Exact test $p = 0.264$; **Figure 3e)**.

For rural living individuals the strongest enriched genus was *Succinivibrio* ($\log_2$FC = 6.76, $p_{adj} = 1.3\times10^{-12}$). Together with *Treponema* ($\log_2$FC = 1.62, $p_{adj} = 0.061$), these genera showed a clear multimodal distribution within rural living individuals, with maximum relative abundance peaks detected at 8.6% and 7.0% for *Succinivibrio* and *Treponema*, respectively (*post-hoc* analysis, **Figure 3f-g**). Six genera belonging to the family of *Prevotellaceae* were strongly associated with rural living, including *Prevotella* (7/9) and *Alloprevotella* ($\log_2$FC $\geq$ 3.92, $p_{adj} \leq 7.7\times10^{-5}$). Similarly, five genera belonging to the family of *Lachnospiraceae* were enriched in rural living individuals including *Butyrivibrio*, *Eubacterium ruminatium* and *Ruminococcus torques* ($\log_2$FC $\geq$ 1.78, $p_{adj} \leq 3.3\times10^{-4}$). Other highly significantly enriched genera in rural individuals included *Fournierella*, *Holdemanella*, *Solobacterium*, *Sutterella*, *Anaeroplasma* and *Catenibacterium* ($p_{adj} \leq 3.4\times10^{-5}$; **Figure 3a** and **Supplementary Figure 5**).

In urban living individuals, abundance of *Bacteroides* and *Parabacteroides* was significantly higher compared to rural living ($\log_2$FC = -3.70 and -2.30, $p_{adj} = 8.9\times10^{-12}$ and 3.78×10^{-7}, respectively). *Bacteroides* showed a multimodal distribution among individuals living at an urban location with two major peaks at a relative abundance of 1.2% and at 24.2% (**Figure 3g**). Other highly urban-associated genera included *Alistipes*, *Parasutterella*, *Bifidobacterium*, *Odoribacter*, *Bilophila* and *Akkermansia* ($p_{adj} \leq 2.8\times10^{-6}$). The gut microbiome of urban living individuals was further enriched for genera belonging to the family of *Enterobacteriaceae*, including *Enterobacter*, *Klebsiella* and *Escherichia*/*Shigella* ($p_{adj} \leq 0.009$; **Figure 3a** and **Supplementary Figure 5**). Taken together, rural living individuals lacked BloSSUM taxa and had

higher abundance of VANISH taxa such as *Prevotella* and *Succinivibrio*, whereas urban individuals were enriched with BloSSUM taxa like *Bacteroides*.

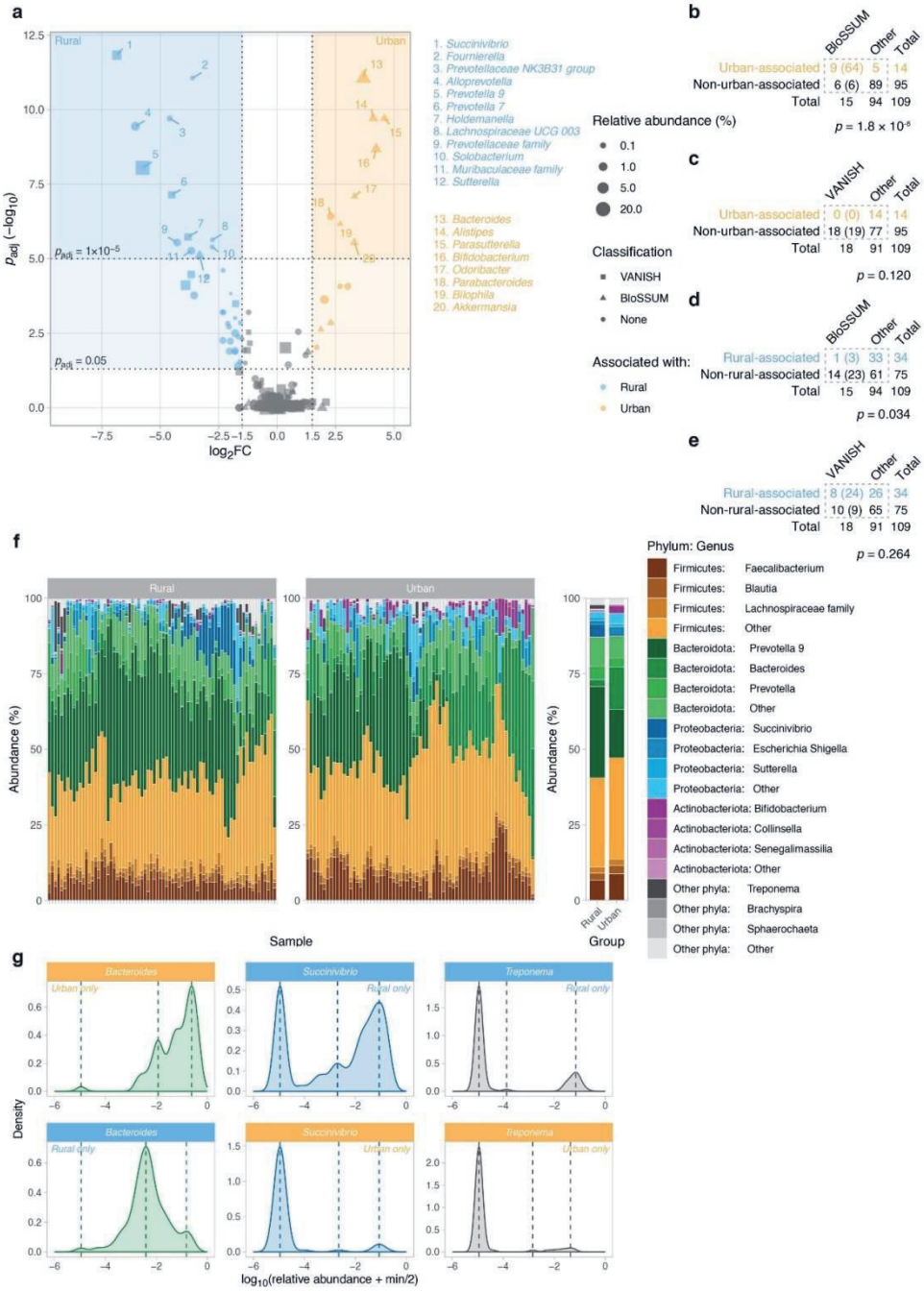

Figure 3 | Differential abundance analyses indicate specific taxa are related to urban/rural living.
a) Volcano plot visualizing genera enriched in rural or urban living individuals. Statistical significance between groups (rural/urban living) was assessed using linear models with genus-level abundance as outcome, adjusting for vaccination status, age and sex. Genera with $p_{adj} < 0.05$ and $\log_2$-fold change (FC) <-1.5 or >1.5 are colored and genera with a $p_{adj} < 1 \times 10^{-5}$ and $\log_2$FC <-1.5 or >1.5 were annotated. The shape of the data points indicates whether these genera were previously considered to belong to VANISH (volatile and/or associated negatively with industrialized societies of humans) or BloSSUM (bloom or selected in societies of urbanization/modernization) taxa. b-e 2 × 2 tables showing the number of BLoSSUM (b and d) or VANISH taxa (c and e) across urban- and rural-associated taxa. The number between brackets indicates the percentage of VANISH/BloSSUM/other taxa out of the total number of (non-)-rural-/urban-associated taxa. Statistical significance was assessed using Fisher's Exact tests. f) Stacked bar chart showing the genus-level taxonomic composition of each sample as relative abundances. Bar colors are based on a hierarchical color palette with hues specified by Phylum and shades specified by Genus. Samples are arranged based on Bray-Curtis dissimilarities and the plot is divided by living location. Right of the per-sample bar chart, a bar chart showing mean relative abundance for each taxon per group is shown. g) Density plots showing the relative abundance distribution of *Bacteroides*, *Succinivibrio* and *Treponema* within rural or urban living individuals. Modes are indicated by dashed lines.

Rural and urban-associated differences in diet are linked to microbiota profiles

Given the multimodal distribution of the relative abundance of specific genera not only between rural and urban living individuals, but also within individuals from the same location (**Figure 3g**), we next assessed the impact of dietary history on microbiota composition. To study this, we clustered samples into two Community State Types (CSTs; **Supplementary Figure 6a** and **Supplementary Figure 6b**) using Dirichlet-multinomial modelling (DMM). CST1 was characterized by a (non-significant) enrichment of VANISH bacteria (25% vs 12%, Fisher's Exact test $p = 0.106$), including *Prevotella*, *Alloprevotella*, *Holdemanella* and *Succinivibrio*, whereas CST2 was strongly enriched for BloSSUM taxa (67% vs 7%, Fisher's Exact test $p = 6.5 \times 10^{-6}$), like *Bacteroides*, *Alistipes*, *Parasutterella* and *Bifidobacterium* (**Figure 4a** and **Supplementary Figure 6c-f**).

Although the majority of samples from rural living individuals clustered in CST1 (93.2%), urban living individuals were split across CST1 (30.4%) and CST2 (69.6%). Baseline characteristics between urban living individuals with CST1 (referred to as 'rural-like urbanites') and urban living individuals with CST2 did not differ, except that

a higher proportion of rural-like urbanites was male (71.5% vs 37.5%, p = 0.009; **Supplementary Table 3**). Comparing rural-like urbanites to urban living individuals revealed highly divergent profiles, with differences similar to those observed when assessing CST1 compared to CST2. Conversely, rural-like urbanites showed much more subtle differences compared to rural living individuals, with enrichment of *Haemophilus* and *Akkermansia,* but lack of rural-associated *Succinivibrio* and *Fournierella* (109 genera tested; $p_{adj} < 0.05$ and $\log_2$-transformed fold change (FC)> 1.5; **Supplementary Figure 7**).

We did not observe differences in either lifestyle or food score between rural-like urban or urban individuals (**Figure 4b**). However, individual food variables did show differences, indicating that rural-like urbanites less frequently consume rice (logistic regression analyses adjusted for all other food variables and sex, β = -1.95, p = 0.032) and more beans and/or peas (β = 0.85, p = 0.078) compared to urbanites belonging to CST2 (i.e. non-rural-like urbanites). Regardless, rural-like urbanites still more frequently consumed rice compared to rural living individuals (β = 2.20, p = 0.033). Furthermore, rural-like urbanites ate significantly less ugali (maize stiff porridge) compared to rural living individuals (β = -3.38, p = 0.023), yet ugali consumption was no different from other urban living individuals (CST2) (β = -0.73, p = 0.212). Last, we found that rural-like urban individuals less frequently eat potatoes compared to both urban living individuals (CST2; β = -0.815, p = 0.087) and rural living individuals (β = -2.02, p = 0.100; **Figure 4c)**. Together, these findings suggest urban living individuals less frequently consuming starch/carbohydrate-rich dietary products (potatoes and rice) and more fiber-rich products (beans and/or peas) harbor a microbiota reminiscent of that of rural living individuals, despite slight differences in dietary habits compared to this latter group.

Genus-level microbiota association tests (linear models including all food variables, vaccination, age and sex) were stratified by living location, given the strong collinearity between living location and dietary habits. Among rural living adults, we detected seven significant associations, suggesting enrichment of *Prevotella¸ Prevotellaceae* and *Eubacterium ventriosum* and lower abundance of *Odoribacter* with increased

consumption of locally brewed beer. *Odoribacter* abundance was also negatively associated with the consumption of bananas, as was *Alistipes* abundance (**Figure 4d)**. Within urban living individuals, we detected five significant associations, indicating frequent ugali consumption is linked to the depletion of *Oscillibacter*, *Christensenellaceae*, *Clostridia, Eubacterium eligens* and *Eubacterium siraeum* (**Figure 4e)**.

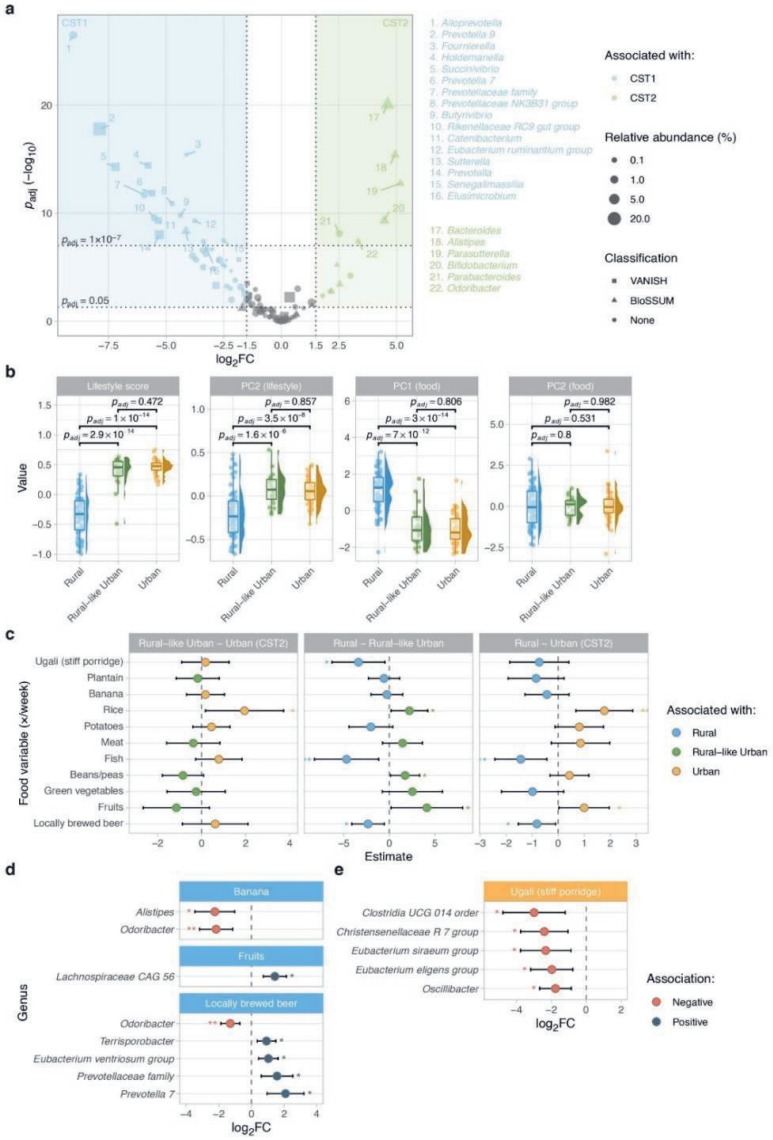

Figure 4 | Frequent consumption of carbohydrate-rich dietary products is associated with urbanization.

a) Volcano plot visualizing genera enriched in CST1 and CST2. Statistical significance between CSTs was assessed using linear models with genus-level abundance as outcome (no adjustment for covariates). Genera with $p_{adj} < 0.05$ and $\log_2$-fold change (FC) <-1.5 or >1.5 are colored and genera with a $p_{adj} < 1 \times 10^{-7}$ and $\log_2$FC <-1.5 or >1.5 were annotated. The shape of the data points indicates whether these genera were previously considered to belong to VANISH (volatile and/or associated negatively with industrialized societies of humans) or BloSSUM (bloom or selected in societies of urbanization/modernization) taxa. b) Differences in lifestyle scores (PC1/PC2; Supplementary Figure 3) and food scores (PC1/PC2; Supplementary Figure 4) between rural living individuals, rural-like urbanites (urban living individuals belonging to community state type [CST]1; Supplementary Figure 6) and urban living individuals (urban living individuals with CST2). See legend Figure 2a-b. Statistical significance between groups ($n = 3$) was assessed using linear models with lifestyle/food score as outcome, adjusting for vaccination status, age and sex. Pairwise contrasts were extracted and adjusted using Tukey's post-hoc test. c) Association between food variables and group (rural living individuals, rural-like urbanites (urban living + CST1) or urban living individuals with CST2). For each comparison (panel), a separate logistic regression model was fitted including all food variables and sex. Model estimates are depicted along the x-axis (colored points). Colors indicate the group with which a given food variable is positively associated. Whiskers denote 95% confidence intervals (CIs; Wald-method). d-e) Association between food variables and genera (present in ≥10% of samples at >0.1% abundance across day 56 samples; $n = 109$) within rural (d) and urban living individuals (e). Results were stratified by food variable and only food variables with any significant ($p < 0.05$) associations are shown. Log_2 fold change (FC) is shown along the x-axis. Whiskers denote 95% confidence intervals (CIs; Wald-method). Asterisks denote statistical significance (*, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.001$).

Rural-like microbiota profile is linked to yellow fever neutralizing antibody titers

Next, we investigated whether vaccine immunogenicity differed between rural and urban living individuals. Across all vaccinated individuals ($N = 155$), no significant differences in yellow fever Plaque Reduction Neutralization Test (PRNT) ($PRNT_{50}$ and $PRNT_{90}$) were found between rural and urban living individuals at 4 weeks post-vaccination (generalized linear mixed effects regression [GLMER], adjusted for age and sex, $p = 0.161$ and $p = 0.226$, respectively, **Supplementary Figure 8a-b)**. However, for the microbiota sub-cohort (i.e. with stool sample available, $N = 121$), yellow fever neutralizing antibodies ($PRNT_{50}$) at 4 weeks post-vaccination were higher in rural (geometric mean titer [GMT], 954 [95% CI, 742 – 1,226]) compared to urban living individuals (656 [95% CI, [490 – 880], $p = 0.042$). This was similar for $PRNT_{90}$ values

(p = 0.032, **Figure 5b** and **Supplementary Figure 8d)**. Antibody titers ($PRNT_{50}$) were lower at 6 months compared to 4 weeks post-vaccination for both urban ($PRNT_{50}$; β = -1.16, p = 0.082) and rural living individuals ($PRNT_{50}$; β = -1.48, p = 0.040), with urban living individuals showing a slightly stronger and statistically significant drop (**Figure 5b)**. Similar results were found when considering all individuals (N = 155; **Supplementary Figure 8c)**.

To assess whether microbiota profiles impact subsequent YF-17D-induced vaccine responses, we first ascertained that vaccination at day 0 had no impact on microbiota profiles collected at day 56. No significant difference in overall microbiota composition between day 56 samples from vaccinated compared to non-vaccinated subjects were found (**PERMANOVA-test, adjusted for living location, age and sex, R^2 = 0.5%, p = 0.787; Figure 2d and Supplementary Figure 9a**). Also, paired day 0 and day 56 samples of vaccinated individuals did not show a consistent direction of movement (**Supplementary Figure 9b-c, PERMANOVA-test, R^2 = 2.3%, p = 0.422**). Within- and between-subject distances between sample pairs and permutation tests across between-subject distances similarly indicated no statistically significant effect of vaccination (**Supplementary Figure 9d-e**). Therefore, day 56 samples were considered representative of day 0 (i.e. before vaccination) and were used as such for downstream analyses.

Next, yellow fever neutralizing antibodies ($PRNT_{50}$ at 4 weeks post-vaccination), were linked to Shannon diversity estimates, adjusting for living location, the interaction between living location and Shannon diversity, age and sex. Shannon diversity was negatively associated with antibody titers in rural-living individuals (**β = -0.345, p = 0.034**), but positively in urban-living individuals (**interaction term; β = 0.496, p = 0.025; Supplementary Figure 8e**). Similar results were found when considering $PRNT_{50}$ at 6 months post-vaccination and PRNT90 titers.

Following, yellow fever neutralizing antibodies ($PRNT_{50}$ or $PRNT_{90}$) were related to CSTs, showing that CST1 (enriched in rural living individuals) is linked to higher antibody titers compared to CST2 (**$PRNT_{50}$; p = 0.005, Figure 5c and Supplementary Figure 8f [$PRNT_{90}$]**). Of note, this association showed large model estimates and was

highly significant, as opposed to initial comparisons between rural and urban living individuals, indicating microbial signatures better explain antibody variation than living location. Though antibody titers were higher 4 weeks post-vaccination, individuals with CST1 showed a much stronger and faster drop over time (**$PRNT_{50}$; β = -1.96,, p = 0.002**) compared to CST2 (**β = -0.02, p = 0.805; Figure 5d)**.

We found that within urban living individuals, there was a striking variation in antibody titers (particularly $PRNT_{50}$) linked to CSTs, with rural-like urbanites showing higher $PRNT_{50}$ compared to urban living individuals with a CST2-profile (**GMT 95%CI, 1,064 [723 – 1,565] vs 548 [381 – 787], p = 0.022, Figure 5e and Supplementary Figure 8g [$PRNT_{90}$]).** Furthermore, in rural-like urbanites, stronger waning was seen (**$PRNT_{50}$ at 6 months compared to 4 weeks post-vaccination; β = -0.502, $p = 2.5 \times 10^{-4}$)** compared to rural living individuals (β = -0.116, p =0.065) and urbanites with CST2-profile (**β = -0.015, p =0.856, Figure 5f**). Findings for $PRNT_{90}$ were similar but less outspoken (**Supplementary Figure 10**). Together, these findings indicate that microbiota profiles might be an important driver of yellow fever neutralizing antibodies in this population.

Differential abundance analyses at genus level (***n* = 109**) revealed no significant associations between specific genera and yellow fever neutralizing antibodies (**$PRNT_{50}$ or $PRNT_{90}$**) at 4 weeks or 6 months post-vaccination (linear models, adjusting for either 1) living location, age and sex or 2) age and sex). This may indicate that the bacterial community in its totality, rather than single genera, relates to yellow fever vaccine immunogenicity.

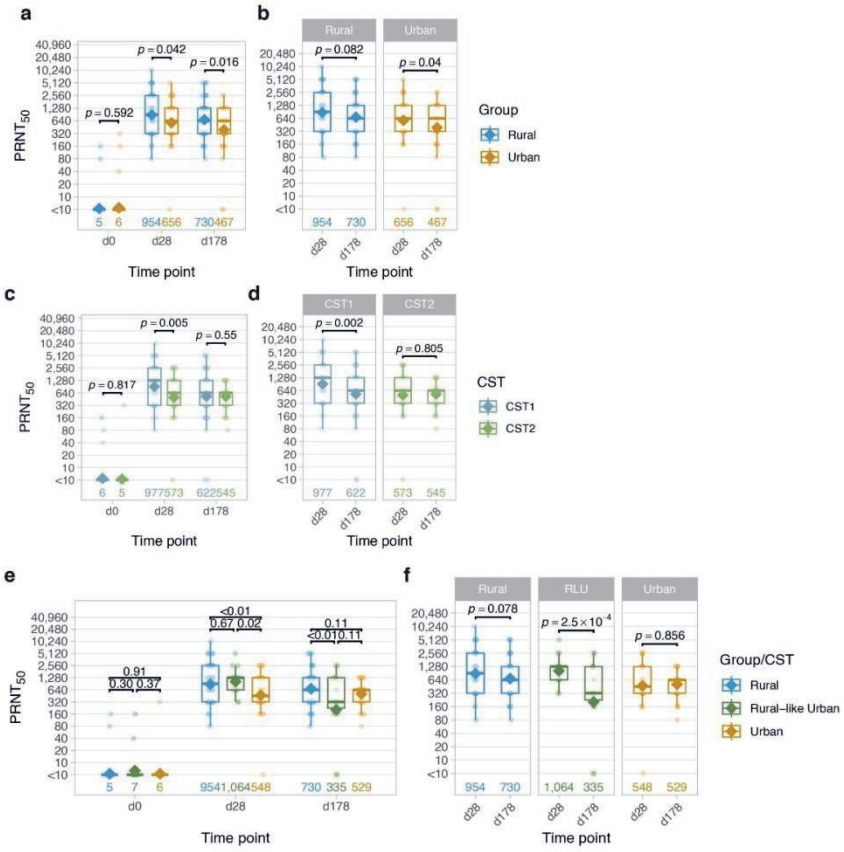

Figure 5 | Yellow fever neutralizing antibody titer variation across living location and with microbiota profiles (*N* = 121). a-f) Boxplots (see legend Figure 2a-b) showing yellow neutralizing antibody titers ($PRNT_{50}$) in vaccinated individuals from whom a stool sample was collected (*N* = 121) at baseline (day 0, d0), 4 weeks post-vaccination (day 28, d28) and 6 months post-vaccination (day 178, d178). a, c, e) Participants were compared according to group (rural/urban living location; a) CST (c) and group/CST (i.e. rural living individuals, rural-like urbanites [CST1] and urban living individuals [CST2]) (e). b, d, f) Boxplots showing yellow neutralizing antibody titers ($PRNT_{50}$) at 4 weeks post-vaccination (day 28, d28) compared to 6 months post-vaccination (day 178, d178) for group (rural/urban living location; b), CST (d) and group/CST (f). Diamond-shaped data points and color values at the bottom of the plot denote log_{10}-base geometric mean titers. Statistical analyses were performed using a generalized linear mixed effects model with log_{10}-transformed $PRNT_{50}$-values as outcome, group, CST or group/CST, time point, the interaction between group, CST or group/CST and time point, age and sex as fixed effects and participant ID as random effects. Pairwise comparisons of estimated marginal means between groups were computed at each time point (a, c and d) or between day 28 and day 178 for each group (b, d and f). Values under the limit of detection (<10) were assumed to have a value of 5.

Discussion

Here, we assessed the associations between rural or urban living location, gut microbiota profiles and yellow fever vaccine responses. We identified striking differences in microbiota community composition between rural and urban living individuals, which appeared in part related to dietary habits. Gut microbiota profile variation was linked to yellow fever vaccine immunogenicity and waning, at least at a microbiota community, but not a genus-level.

We show that rural living individuals harbor a gut microbiota enriched for *Succinivibrio*, *Treponema* and *Prevotella*, which is consistent with literature[20,26,28,39], and likely explained by variation in dietary habits. Higher abundance of these microbiota members has been associated with the digestion of plant-rich diets and the production of short-chain fatty acids[24,40,41]. Indeed, we found that Tanzanians living in rural settings consume more grain-based food products such as ugali (maize stiff porridge), vegetables and local beer made by a mix of fermented bananas and finger millet[26]. Strikingly, within those living in rural areas, local beer consumption in particular was associated with a higher abundance of *Prevotella*, suggesting it may be a particularly important driver of the rural microbiota signature.

Urban living individuals exhibited a higher abundance of *Bacteroides*, *Parabacteroides*, *Enterobacteriaceae* and *Bifidobacterium,* which may be related to the consumption of a more carbohydrate-rich diet, again largely aligning with literature[27,31,42]. Interestingly, approximately half of the urban living individuals showed a high abundance of *Prevotella*, which we identified as characteristic of the rural microbial signature. The microbiota composition of this subgroup of urban living individuals co-clustered with rural living individuals based on their microbial make-up. Despite that, these rural-like urbanites showed a different dietary consumption pattern from both rural living individuals and their urban counterparts, with high consumption of beans and peas (rich in proteins, carbohydrates and dietary fibers) and lower rates of potato and rice consumption. Within the urban population we found that frequent

consumption of ugali (maize stiff porridge) is related to a reduced abundance of *Eubacterium siraeum*. *Eubacterium siraeum* has previously been linked to the consumption of a Western diet and systemic inflammation[43], possibly indicating that ugali consumption limits the establishment of microbes related to industrialization.

Genera associated with rural or urban living location were classified as either BloSSUM or VANISH taxa. The distinction between these taxa has been based on previous work comparing the gut microbiome of a Tanzanian population of Hadza hunter-gatherers to that of populations living industrialized lifestyles[22]. Although rural living individuals in our study showed a lack of industrialized lifestyle-associated BloSSUM taxa, we did not observe a significant enrichment for VANISH taxa, suggesting that indeed the population of rural living individuals recruited in our study is possibly in transition between traditional and industrialized lifestyles. Conversely, a clear signature of Westernized microbiota profiles was detected in urban living individuals, indicated by significant enrichment of BloSSUM taxa. Apart from diet, there may be other host and environmental factors contributing to the observed differences in microbiota composition between rural and urban living individuals. Among others, agricultural activities, such as livestock farming[44,45], and environmental exposures, including indoor cooking, wood stove cooking or exposure to pollutants, may impact microbiota composition[46]. Helminth infections have previously been linked to microbiota changes[47-50], but since helminth infection prevalence was low in our rural cohort (4.3%), we presume this was not a main driver of rural urban differences in our study. We detected slightly higher yellow fever neutralizing antibody responses 4 weeks post-vaccination in participants living in rural compared to urban settings. The difference was statistically significant in the microbiota sub-cohort, but not in the total vaccinated cohort. At 6 months, titers remained higher in rural settings. This is not in line with the general hypothesis that vaccine immunogenicity in rural populations is lower than urban urban-living individuals, especially within LMICs, which has been observed for a wide range of vaccines[6,51]. Yellow fever vaccine, which is highly effective, has previously been used to model human immune responses to vaccines in general. As such, several studies assessed yellow fever vaccine immunogenicity, showing lower antibody titers and rapid

waning in Ugandan compared to Swiss individuals and lower seroprevalence rates in rural Ghanaian compared to urban Malian infants[52,53]. In both studies, the observed differences in immunogenicity are relatively small compared to other vaccines, such as for malaria[54,55], and may be obscured by between-country differences. Moreover, discordant results have been reported in some studies assessing factors associated with yellow fever vaccine immunogenicity[56,57]. Regardless, a recent study in Uganda (POPVAC)[58], did show higher yellow fever vaccine antibody titers in urban living individuals compared to individuals living in rural settings, which is in contrast with our findings and at least in part could be explained by the high helminth and malaria infection prevalence in the Ugandan study. Also, the rural sites sampled in our study are relatively developed compared to other rural sites in Tanzania and some other East African countries, with better access to health care and nutrition[59,60]. Last, rural individuals in our study live at high altitude, which induces a hypoxic state, as reflected by their high hemoglobin levels. We speculate this causes an increase of transcriptional factor hypoxia-inducible factor (HIF), which induces metabolic and phenotypic changes in B cells and boosts B cell differentiation and antigen switching, thus resulting in higher antibody titers in these rural individuals[61-64]. We found that especially within urban living individuals, a rural-like microbiota profile was related to relatively higher antibody titers, but stronger waning, as opposed to a more *Prevotella*-depleted, industrialized microbial community composition. Despite this, no significant associations were detected between specific genera and yellow fever vaccine-induced neutralizing antibody titers. This lack of genus-level associations could be explained by insufficient statistical power or limited sequencing resolution from 16S-rRNA-sequencing. Alternatively, it may suggest that the overall bacterial community, rather than individual genera, is linked to yellow fever vaccine immunogenicity. The microbiota has been suggested to potentiate vaccine responses by providing adjuvating signals, although this seems most relevant to vaccines other than yellow fever vaccine, which has endogenous adjuvants and is therefore able to engage with toll-like receptors (TLRs) and RIG-1 receptors itself[32]. Alternatively, we speculate higher yellow fever antibody titers observed in rural living individuals and rural-like urbanites could be related to increased consumption of fiber-rich diets which enriches for short-chain fatty acid (SCFA)-producing bacteria, such as *Prevotella[40].* In B cells, SCFAs increase

acetyl-CoA and metabolic activity, resulting in increased antibody production[65]. Indeed, a study showed that mice fed with a diet low in dietary fibers had lower production of SCFAs and a reduced specific antibody response towards pathogens[65]. It is possible that the microbiota-associated increase in antibody titers observed in rural individuals in our study has been obscured in other rural populations by factors such as a high prevalence of helminth infections and other infections such as malaria.

Although the gut microbiota of rural and urban living individuals in LMICs has been described previously, there remains a significant gap in the literature regarding gut microbiota profiles from LMIC populations currently transitioning from traditional to industrialized lifestyles. Our work expands on this topic, but also furthers our understanding on microbiome-host associations in the context of yellow fever vaccination. Our study also has limitations, such as the use of 16S-rRNA sequencing rather than shotgun sequencing. Additionally, as this was primarily an observational study aimed at identifying associations between the gut microbiome and the yellow fever vaccine responses, we could not assess causal effects. Also, we cannot exclude the possibility that (unmeasured) microbiota-independent factors (e.g. altitude, genetic variation and historic microbial exposures) drive vaccine immunogenicity, although the observed variation within the urban subgroup advocates against this. Fungal and viral microbiota may covary with the bacterial microbiota and explain the residual variation in vaccine responses we identified. We lacked longitudinal stool samples for most participants, as most samples were collected post-vaccination, which may have influenced the findings. However, our extensive analyses, also leveraging the paired samples we had available, indicated no major impact of the vaccine on the gut microbiota. This warrants our assumption that the microbiota measured post-vaccination is reflective of the baseline microbiota in these individuals.

To further explore microbe-host interactions in the context of yellow fever vaccination, future studies could include measurements of the cellular immune responses, which have previously been shown to differ at least between HIC and LMIC-populations[52]. Taken together, we show strong differences in gut microbiota profiles between rural and urban living individuals, with part of urban living individuals currently

transitioning towards a more industrialized microbiota profile. Microbiota variation within urban living individuals was associated with moderate variation in yellow fever vaccine-induced neutralizing antibodies and antibody waning. These findings suggest that gut microbiota profiles may impact vaccine responsiveness in a vaccine- and context-dependent manner. Identifying adult subpopulations where microbiota influences vaccine responsiveness creates opportunities for research on microbiota-based interventions.

Methods

Study design

This is a prospective longitudinal cohort study (CapTan) in a healthy, 18-35-year-old Tanzanian population recruited in a rural and urban Moshi, Northern Tanzania. Volunteers were enrolled and randomized into a vaccine group receiving a single 0.5 mL intramuscular dose of the yellow fever vaccine (YF-17D, Sanofi-Pasteur) and a control group. Randomization occurred by allocating every sixth individual to the control group. No placebo was administered to the control group. Neither the volunteers nor study personnel were blinded to group allocation. Plasma samples were collected at baseline (pre-vaccination), day 28 (4 weeks post-vaccination), 56 (8 weeks post-vaccination) and 178 (~6 months post-vaccination). Paired stool samples were collected at baseline and on day 56 for 11 individuals. For 143 individuals, stools samples were collected on day 56 only. All questionnaires and clinical samples were collected by a well-trained study team consisting of medical doctors, nurses and laboratory scientists. All samples were processed according to established standard operational procedures and good clinical and laboratory practice principles.

The study protocol was approved by the Ethical Board of the Kilimanjaro Christian Medical University College (No. 2588) and by the Tanzania National Ethical Committee Board (NIMR/HQ/R.8a/Vol.IX/4089). The study was registered under The Pan African Clinical Trial Registry (PACTR) with trial number PACTR202405738173023 on 03 May 2024. Data were collected in REDCap, a cloud-

based electronic data collection system, with a server hosted at the Kilimanjaro Clinical Research Institute (KCRI) in Tanzania.

Description of study areas

The study was conducted in rural and urban Moshi located in the Kilimanjaro region (total population of 1.9 million (**Figure 1a)**. The rural study area is at an elevation of 2,000-2,100 meters above sea level, while urban Moshi is at 700-950 meters above sea level. The district of Moshi City (urban Moshi) is the administrative, commercial and educational center of the Kilimanjaro region, having 331,733 inhabitants. Most people practice a Western lifestyle with good general sanitation and access to clean water. The main ethnicities are Chagga and Pare. Formal business is the main activity, followed by government and public employment, while a small proportion of people are involved in agricultural and entrepreneurial activities. Rural Moshi has about 535,803 inhabitants who are mainly involved in farming activities. Most people have access to clean water, but a small proportion use borehole water sources. People live in large family units, and their main economic activities are subsistence farming and animal husbandry. The primary ethnicity is Chagga, and people follow Chagga traditions, such as drinking local brews made from banana or plantain.

Participant screening and enrollment

In rural Moshi, study information was given through community leaders and announcements during mass gatherings in mosques, churches and village meetings. In urban Moshi, study information was distributed using leaflets and through community leaders, office announcements and university gatherings. Eligible participants (aged 18-35 years and with permanent residency of a given location) were asked to enroll in the study. Following informed consent, 233 participants were voluntarily screened for in- and exclusion criteria. Exclusion criteria were having an acute or chronic disease (including HIV, tuberculosis, cancer, cardiovascular disease, gastrointestinal diseases, recurrent infections, liver, renal, endocrine or neurological disorders), ≥2 hospital admissions times/year in the last year, chronic use of antibiotics or corticosteroids, use

of other immunosuppressive drugs, recent receipt of another vaccine, lactation, positive pregnancy test, history of blood product transfusion, hemoglobin level ≤8.5 g/dL, testing positive HIV or malaria, low or high blood pressure (≤90/60mmHg and ≥140/90mmHg, respectively) or high blood glucose (≥7.1mmol/L fasting or ≥11.1mmol/L random glucose).

Participants were screened for HIV infection (SDBIOLINE HIV-1/2 3.0kit, LOT:03ADG020A), malaria (Malaria Ag p.f/Pan, Ref: 05FK60, LOT:05EDG018A) and soil-transmitted helminth such as hookworms (*Ancylostoma duodenale* and *Necator americanus*), *Trichuris trichiura*, *Ascaris lumbricoides*, *Strongyloides stercoralis* and *Schistosoma mansoni* using Kato-Katz or POC-CCA (*Schistosoma mansoni* and *Schistosoma haematobium*; butch no:220701075). Furthermore, hemoglobin levels were measured (HemoCue Hb 301(CE:1450820055) and random blood glucose was assessed (ACCU-CHECK glucose test strips, Roche Diabetic care,06993761001). Weight and height were measured using a well-calibrated machine (RGZ-160, made in China), and blood pressure was measured using OMRON(SN:202111007949V). All individuals with abnormal laboratory or clinical findings except those with parasitic infections received nurse counselling, referral, or treatment before being excluded. Based on exclusion criteria, 48 of 233 participants were excluded.

Lifestyle questionnaire

Questionnaires were adapted from the Tanzania Demographic and Health Survey and Malaria Indicator Survey (TDHS-MIS) and a food history questionnaire previously applied to a similar population[54,60]. We collected information on the water source, toilet type, available cooking facilities and the materials used to construct the floor, roof and walls of the house. We assessed exposure to livestock by collecting information on the number of milk cows, cattle, goats, sheep, horses and poultry owned. Inquiries were made on land ownership and possession of non-productive assets, including radios, televisions, computers, refrigerators, ironing tools (whether powered by charcoal or electricity), watches, motorcycles, trucks, animal-drawn carts,

generators and motorboats. Using food questionnaires, data was collected on the frequency of dietary products participants consume weekly, including ugali (maize stiff porridge), plantain, banana, rice, potatoes, meat, fish, beans/peas, green vegetables, fruits and locally brewed beer.

Microbiota profiling

Stool sample collection

Stool samples were collected at baseline and/or at day 56. For rural individuals, stool samples were collected at the outpatient clinic or at home. For urban individuals, stool was collected at the KCMC or at home. Feces were initially stored in a dry stool container, before it was transferred into DNA/RNA Shield Fecal Collection (Zymo Research, Irvine, California, USA) for transportation and storage (median [IQR] time between stool production and storage in medium, 58.5 [15.0-113.0] minutes). Samples were stored in a -80°C freezer at the KCMC (median [IQR] time between storage in medium and storage in freezer, 120.5 [93.0-168.5 minutes]). Samples were transported to The Netherlands on dry ice and stored at -80°C before further processing. Samples of individuals who took antibiotics between study enrollment and stool sample collection at day 56 were excluded (n = 9).

Bacterial DNA isolation

DNA was extracted from 250 µL diluted feces (in DNA/RNA Shield) by Repeated Bead Beating (RBB) combined with purification using the chemagic DNA Stool 200 Kit H96 (Revvity, Waltham, Massachusetts, USA). Briefly, 250 µL diluted feces was homogenized with 1.0 mL Lysis Buffer 1 of the chemagic DNA Stool 200 Kit H96 by vortexing in a 2 mL tube (with screw cap) containing 0.5 g of sterile zirconia beads Ø 0.1mm (BioSpec, Cat. No. 11079101z). Cells were mechanically lysed on a FastPrep-24™ 5G Instrument (MP Bio, Irvine, California, USA) at 5.5 m/s for 3 cycles of 1 minute. Samples were subsequently centrifuged (16,000 x g 4°C, for 5 min) and the supernatant was transferred to a new tube to which 30 µL Proteinase K was added, mixed and incubated for 10 minutes at 70°C. Thereafter, samples were incubated for another 5 minutes at 95°C followed by centrifugation for 5 minutes at high speed

(13,000 rpm). Deep-well plates were filled with 800 µL of the supernatant (lysates) and further isolation was done on a chemagic™ 360 instrument (Revvity, Waltham, Massachusetts, USA) according to the manufacturer's instructions. Finally, purified DNA was quantified on a Qubit Fluorometer (Thermofischer Scientific, Waltham, Massachusetts, USA) using the dsDNA HS Assay Kit (Invitrogen, Waltham, Massachusetts, USA).

16S-rRNA gene amplicon sequencing

The variable regions of the V3-V4 of the bacterial 16S rRNA gene were PCR amplified from each DNA sample in a single reaction workflow with simultaneous indexing and target amplification using the EasySeq™ 16S Microbiome Library Prep Kit (NimaGen, Nijmegen, the Netherlands) according to the manufacturer's instructions. Amplicon libraries were sequenced on an Illumina MiSeq instrument (Illumina, Eindhoven, The Netherlands) (MiSeq Reagent Kit v3, 2 × 300 cycles, 10% PhiX) to generate paired-end reads of 300 bases in length in both directions.

16S rRNA gene amplicon sequence data processing

Forward and reverse primers were removed using cutadapt v4.7[66]. Following, using DADA2 (v1.28.0) paired-end sequences were filtered and trimmed (maxEE = 2, truncLen = 240/210bp), denoised, merged (minOverlap = 10, maxMismatch = 0) and a sequence table was constructed. Chimeras were identified and removed (method = 'consensus'). After denoising and merging, any ASVs with a length of <350 or >500 bases were discarded. ASVs were annotated up to genus-level using the DADA2 implementation of the naïve Bayesian classifier based on the SILVA v138.1 reference database. Species-level annotations were added using the *addSpecies()*-function[67]. ASVs not assigned to the kingdom Archaea/Bacteria or annotated as Mitochondria (family) or Chloroplast (class) were removed.

Yellow fever neutralizing antibody titer measurements

Blood sample collection and processing

Blood samples were collected were collected in 5mL EDTA tubes on day 0 (pre-vaccination), day 28 (4 weeks post-vaccination; presumed peak in antibody titers) and

day 178 (~6 months post-vaccination; detect delayed response/assess weaning status). Blood samples were transported to a clinical laboratory for plasma separation through centrifugation. Plasma samples were stored at -80°C within two hours. Samples were shipped between institutions on dry ice.

Yellow fever plaque reduction neutralization tests

Plasma neutralizing antibodies against the yellow fever virus from all individuals were quantified using plaque reduction neutralization tests (PRNTs). Results are presented as $PRNT_{50}$ and $PRNT_{90}$ titers, which correspond to the reciprocal of the plasma dilution, achieving a 50% and 90% reduction in virus plaque-forming units per 0.1 mL of the reference 17D virus preparation, respectively. Assay quality control included determining the 50% and 90% neutralization cut-off values for each assay via back titration of the virus inoculum. Further details are provided elsewhere[68].

Data analysis

All data preprocessing and statistics were performed in R v4.3.3 within R Studio v2024.04.1+748 (Boston, MA). Microbiota-specific analyses and visualization, including alpha- and beta-diversity analyses and microbiota association tests were performed using the microViz v0.12.1 R package[69]. All statistical tests were two-sided and *p*-values were corrected for multiple testing using the Benjamini-Hochberg procedure (referred to as p_{adj}-values). *P*-/p_{adj}-values<0.05 were considered statistically significant.

Baseline characteristics

Descriptives for baseline and lifestyle questionnaire data were generated using the gtsummary v1.7.2 R package.

Lifestyle and food score

Lifestyle questionnaire data (N = 185) were mode imputed (missing values) and subjected to either Multiple Correspondence Analysis (MCA) or Principal Component Analysis (PCA) to calculate lifestyle and food score, respectively. Lifestyle score was calculated by applying MCA to categorical questionnaire data (n = 38 manually curated

lifestyle-related questions; 21 on assets, 11 on food and 6 on housing) (FactoMineR v2.7 R package, *MCA()*-function; PC1 defined as 'lifestyle score'). (Cumulative) variable category contributions were extracted and shown. To calculate a food score, variables capturing the frequency of consumption of dietary products (n = 11; 0, 1, 2-4 or ≥5×/week) were converted into ordinal variables and used as input variables for a PCA ordination (*PCA()*-function). Coordinates of samples and variable categories/variables were visualized in biplots. For lifestyle score analyses, (cumulative) variable category contributions were extracted and shown.

Alpha-diversity analysis

Diversity analyses were performed on unfiltered and non-rarefied raw read counts (ASVs present in ≥2 samples). Shannon diversity was primarily used as a measure for within-sample/alpha-diversity, leveraging both species richness and evenness (ASV- and genus-level). In addition, we compared the number of observed ASVs or genera. Statistical significance between groups was assessed using linear models with observed richness or Shannon diversity as outcome, adjusting for vaccination status, age, sex and sequencing depth.

Beta-diversity analysis

Beta-diversity analyses were performed on centered-log-ratio [CLR]-transformed genus-level abundance matrices. CLR-transformed abundances were used in principal components analysis (PCA) to visualize major patterns of microbiota variation. Associations between rural/urban living location, host and environmental variables (age, sex, helminth infection status, highest level of education, body mass index (BMI), lifestyle and food scores (PC1 and PC2), vaccination status and technical variables (sequencing depth) were explored by multivariable Permutational Multivariate Analysis of Variance (Aitchison-distance matrix; PERMANOVA), adjusting for living location, vaccination, age and sex as appropriate. For variables for which collinearity with living location was expected (i.e. lifestyle/food scores, helminth infection status and sex), models were adjusted for vaccination, age and sex (dropping living location). Paired day 0 and day 56 samples from vaccinated individuals were tested for

differences between time points (indicating a vaccination effect) using a PERMANOVA with permutations restricted within participant.

Microbiota clustering

Microbiota profiles (n = 154 samples) were clustered into Community State Types (CSTs) by fitting a Dirichlet Multinomial model (DirichletMultinomial v1.46.0 R-package; *dmn()*-function). Genus-level count data were taken as input and models were fitted with 1 to 10 components. The optimal number of Dirichlet components was determined by inspecting measures of fit (Laplace, AIC and BIC). Species associated with either CST1 or CST2 were identified using differential abundance analyses (MaAsLin2; no adjustment for covariates, see below).

Food-variable association tests

To assess the association between food variables (frequency of consumption of dietary products per week) and living location and living location/CST (including rural-like urban individuals), we used logistic regression analysis, adjusting for all other food variables and sex.

Differential abundance analysis

Differentially abundant taxa between groups were identified using linear models, like the implementation in the MaAsLin2 framework (default parameters; $\log_2$-normalisation)[68]. For each comparison genera present at >0.1% relative abundance in ≥10% of samples were selected. We explored the associations between 1) rural/urban living location (adjusted for vaccination status, age and sex), 2) CST1 and CST2 (no adjustment for covariates), 3) urban (urban + CST2)/rural living individuals and rural-like urbanites (urban + CST1) (no adjustment for covariates), 4) food variables (stratified by living location; adjusted for other food variables and sex), and 5) $\log_{10}$-transformed yellow fever neutralizing antibodies (adjusted for living location, age and sex or age and sex) and relative abundances of individual taxa. When comparing >2 groups (e.g. urban/rural/rural-like urbanites), estimates and p-values for each pairwise comparison between groups (e.g. urban vs rural) were extracted using the *emmeans* v1.8.5 R-package. p-values were adjusted per rank, term and contrast, as appropriate,

and resulting BH-adjusted *p*-values of below 0.05 were considered statistically significant.

BloSSUM and VANISH taxa

Classification into BloSSUM (bloom or selected in societies of urbanization/modernization) and VANISH (volatile and/or associated negatively with industrialized societies of humans) taxa was based on a publication describing gut microbiota profiles in a non-industrialized population of Hadza hunter-gatherers. Lists of BloSSUM and VANISH taxa were extracted from the publication and collapsed at genus-level. Genera classified as both BloSSUM- and VANISH-associated were dropped. For differentially abundant genera associated with rural/urban living or CST1/CST2, we tested for significant enrichment of either BloSSUM or VANISH taxa using Fisher's Exact tests.

Multimodal analyses

Based on visual inspection of stacked bar plots, we manually selected several genera for which the (multimodal) distribution of relative abundance was assessed in a post-hoc analysis using the multimode R-package. The number of modes was determined using the *nmodes()*-function, based on inspection of a stairs plot across a range of density bandwidths. Mode location was determined by the *locmodes()*-function.

Yellow fever neutralizing antibody titers

Log_{10}-base geometric mean yellow fever neutralizing antibody titers ($PRNT_{50}$ and $PRNT_{90}$) at baseline (pre-vaccination), day 28 (4 weeks post-vaccination) and at day 178 (~6 months post-vaccination) were calculated. Values under the limit of detection (<10) were assumed to have a value of 5. Statistical analyses were performed using a generalized linear mixed effects model with log_{10}-transformed $PRNT_{50}$/$PRNT_{90}$-values as outcome, group, CST or group/CST, time point, the interaction between group, CST or group/CST and time point, age and sex as fixed effects and participant ID as random effects. Pairwise comparisons of estimated marginal means between groups were

computed at each time point or between day 28 and day 178 for each group (emmeans v1.10.0 R package).

Data availability

16S-rRNA sequencing data from this study are available from NCBI under BioProject accession number PRJNA1141956. Other data are available from the corresponding author upon reasonable request.

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Authors contributions

Conceptualization, J.J.P., M.M.A.R.v.D., M.Y.; Methodology, J.J.P., M.M.A.R.v.D., S.E.M., S.P.J., P.C.W.H., L.W., M.Y.; Data Curation, J.J.P., D.B., W.A.A.d.S.P.; Formal Analysis, J.P., K.S., J.J.P., D.B., M.M., M.Y., W.A.A.d.S.P.; Investigation, J.J.P., M.M.A.R.v.D., N.A., A.N., Y.K., M.K.; Writing - Original Draft, J.J.P., M.Y., W.A.A.d.S.P; Review & Editing, W.A.A.d.S.P., M.M., J.P., K.S., V.I.K., E.R.S., A.M., S.E.M., S.P.J., P.C.W.H., L.W., M.Y.; Supervision, J.P., S.P.J, S.E.M., P.C.W.H., W.A.A.d.S.P., M.Y.; Funding Acquisition, M.Y. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interest.

References

1. Montero DA, Vidal RM, Velasco J, et al. Two centuries of vaccination: historical and conceptual approach and future perspectives. *Front Public Health*. 2024;11. doi:10.3389/fpubh.2023.1326154
2. Alghamdi S. The role of vaccines in combating antimicrobial resistance (AMR) bacteria. *Saudi J Biol Sci*. 2021;28(12):7505-7510. doi:10.1016/j.sjbs.2021.08.054
3. Sinumvayo JP, Munezero PC, Tope AT, et al. Vaccination and vaccine-preventable diseases in Africa. *Sci Afr*. 2024;24:e02199. doi:10.1016/j.sciaf.2024.e02199
4. Micoli F, Bagnoli F, Rappuoli R, Serruto D. The role of vaccines in combatting antimicrobial resistance. *Nat Rev Microbiol*. 2021;19(5):287-302. doi:10.1038/s41579-020-00506-3
5. Greenwood B. The contribution of vaccination to global health: past, present and future. *Philosophical Transactions of the Royal Society B: Biological Sciences*. 2014;369(1645):20130433.doi:10.1098/rstb.2013.0433
6. Lynn DJ, Benson SC, Lynn MA, Pulendran B. Modulation of immune responses to vaccination by the microbiota: implications and potential mechanisms. *Nat Rev Immunol*. 2022;22(1):33-46. doi:10.1038/s41577-021-00554-7
7. Nkurunungi G, Nassuuna J, Natukunda A, et al. The effect of intensive praziquantel administration on vaccine-specific responses among schoolchildren in Ugandan schistosomiasis-endemic islands (POPVAC A): an open-label, randomised controlled trial. *Lancet Glob Health*. 2024;12(11):e1826-e1837. doi:10.1016/S2214-109X(24)00280-8
8. van Riet E, Retra K, Adegnika AA, et al. Cellular and humoral responses to tetanus vaccination in gabonese children. *Vaccine*. 2008;26(29-30):3690-3695. doi:10.1016/j.vaccine.2008.04.067
9. Riet E van, Adegnika AA, Retra K, et al. Cellular and Humoral Responses to Influenza in Gabonese Children Living in Rural and Semi-Urban Areas. *J Infect Dis*. 2007;196(11):1671-1678. doi:10.1086/522010
10. Lynn DJ, Pulendran B. The potential of the microbiota to influence vaccine responses. *J Leukoc Biol*. 2018;103(2):225-231. doi:10.1189/jlb.5MR0617-216R
11. Zimmermann P, Curtis N. Factors That Influence the Immune Response to Vaccination. *Clin Microbiol Rev*. 2019;32(2). doi:10.1128/CMR.00084-18
12. Falahi S, Kenarkoohi A. Host factors and vaccine efficacy: Implications for COVID-19 vaccines. *J Med Virol*. 2022;94(4):1330-1335. doi:10.1002/jmv.27485
13. Ng SC, Peng Y, Zhang L, et al. Gut microbiota composition is associated with SARS-CoV-2 vaccine immunogenicity and adverse events. *Gut*. 2022;71(6):1106-1116. doi:10.1136/gutjnl-2021-326563
14. Huda MN, Lewis Z, Kalanetra KM, et al. Stool Microbiota and Vaccine Responses of Infants. *Pediatrics*. 2014;134(2):e362-e372. doi:10.1542/peds.2013-3937
15. Huda MN, Ahmad SM, Alam MJ, et al. Bifidobacterium Abundance in Early Infancy and Vaccine Response at 2 Years of Age .

Pediatrics. 2019;143(2). doi:10.1542/peds.2018-1489

16. Harris V, Ali A, Fuentes S, et al. Rotavirus vaccine response correlates with the infant gut microbiota composition in Pakistan. *Gut Microbes*. 2018;9(2):93-101. doi:10.1080/19490976.2017.1376162

17. Fix J, Chandrashekhar K, Perez J, et al. Association between Gut Microbiome Composition and Rotavirus Vaccine Response among Nicaraguan Infants. *Am J Trop Med Hyg*. 2020;102(1):213-219. doi:10.4269/ajtmh.19-0355

18. Harris VC, Armah G, Fuentes S, et al. Significant Correlation Between the Infant Gut Microbiome and Rotavirus Vaccine Response in Rural Ghana. *J Infect Dis*. 2017;215(1):34-41. doi:10.1093/infdis/jiw518

19. Ray S, Narayanan A, Vesterbacka J, et al. Impact of the gut microbiome on immunological responses to COVID-19 vaccination in healthy controls and people living with HIV. *NPJ Biofilms Microbiomes*. 2023;9(1):104. doi:10.1038/s41522-023-00461-w

20. Schnorr SL, Candela M, Rampelli S, et al. Gut microbiome of the Hadza hunter-gatherers. *Nat Commun*. 2014;5(1):3654. doi:10.1038/ncomms4654

21. Smits SA, Leach J, Sonnenburg ED, et al. Seasonal cycling in the gut microbiome of the Hadza hunter-gatherers of Tanzania. *Science (1979)*. 2017;357(6353):802-806. doi:10.1126/science.aan4834

22. Carter MM, Olm MR, Merrill BD, et al. Ultra-deep sequencing of Hadza hunter-gatherers recovers vanishing gut microbes. *Cell*. 2023;186(14):3111-3124.e13. doi:10.1016/j.cell.2023.05.046

23. Yatsunenko T, Rey FE, Manary MJ, et al. Human gut microbiome viewed across age and geography. *Nature*. 2012;486(7402):222-227. doi:10.1038/nature11053

24. Ayeni FA, Biagi E, Rampelli S, et al. Infant and Adult Gut Microbiome and Metabolome in Rural 25. Bassa and Urban Settlers from Nigeria. *Cell Rep*. 2018;23(10):3056-3067. doi:10.1016/j.celrep.2018.05.018

25. Das B, Ghosh TS, Kedia S, et al. Analysis of the Gut Microbiome of Rural and Urban Healthy Indians Living in Sea Level and High Altitude Areas. *Sci Rep*. 2018;8(1):10104. doi:10.1038/s41598-018-28550-3

26. Stražar M, Temba GS, Vlamakis H, et al. Gut microbiome-mediated metabolism effects on immunity in rural and urban African populations. *Nat Commun*. 2021;12(1):4845. doi:10.1038/s41467-021-25213-2

27. van der Vossen EWJ, Davids M, Bresser LRF, et al. Gut microbiome transitions across generations in different ethnicities in an urban setting—the HELIUS study. *Microbiome*. 2023;11(1):99. doi:10.1186/s40168-023-01488-z.

28. Rampelli S, Schnorr SL, Consolandi C, et al. Metagenome Sequencing of the Hadza Hunter-Gatherer Gut Microbiota. *Current Biology*. 2015;25(13):1682-1693. doi:10.1016/j.cub.2015.04.055

29. 30.Maghini DG, Oduaran OH, Wirbel J, et al. Expanding the human gut microbiome atlas of Africa. Published online March 14, 2024. doi:10.1101/2024.03.13.584859

30. Tamburini FB, Maghini D, Oduaran OH, et al. Short- and long-read metagenomics of urban and rural South African gut microbiomes reveal a transitional composition and undescribed taxa. *Nat Commun*. 2022;13(1):926. doi:10.1038/s41467-021-27917-x

31. Ramaboli MC, Ocvirk S, Khan Mirzaei M, et al. Diet changes due to urbanization in South Africa are linked to microbiome and metabolome signatures of Westernization and colorectal cancer. *Nat Commun*. 2024;15(1):3379. doi:10.1038/s41467-024-46265-0

32. Oh JZ, Ravindran R, Chassaing B, et al. TLR5-Mediated Sensing of Gut Microbiota Is Necessary for Antibody Responses to Seasonal Influenza Vaccination. *Immunity*. 2014;41(3):478-492. doi:10.1016/j.immuni.2014.08.009

33. Tang B, Tang L, He W, et al. Correlation of gut microbiota and metabolic functions with the antibody response to the BBIBP-CorV vaccine. *Cell Rep Med*. 2022;3(10):100752. doi:10.1016/j.xcrm.2022.100752

34. Pabst O, Hornef M. Gut Microbiota: A Natural Adjuvant for Vaccination. *Immunity*. 2014;41(3):349-351. doi:10.1016/j.immuni.2014.09.002

35. Lynn MA, Tumes DJ, Choo JM, et al. Early-Life Antibiotic-Driven Dysbiosis Leads to Dysregulated Vaccine Immune Responses in Mice. *Cell Host Microbe*. 2018;23(5):653-660.e5. doi:10.1016/j.chom.2018.04.009

36. Nyangahu DD, Happel AU, Wendoh J, et al. Bifidobacterium infantis associates with T cell immunity in human infants and is sufficient to enhance antigen-specific T cells in mice. *Sci Adv*. 2023;9(49). doi:10.1126/sciadv.ade1370

37. Nadeem S, Maurya SK, Das DK, Khan N, Agrewala JN. Gut Dysbiosis Thwarts the Efficacy of Vaccine Against Mycobacterium tuberculosis. *Front Immunol*. 2020;11. doi:10.3389/fimmu.2020.00726

38. De Filippo C, Cavalieri D, Di Paola M, et al. Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. *Proceedings of the National Academy of Sciences*. 2010;107(33):14691-14696. doi:10.1073/pnas.1005963107

39. Gomez A, Petrzelkova KJ, Burns MB, et al. Gut Microbiome of Coexisting BaAka Pygmies and Bantu Reflects Gradients of Traditional Subsistence Patterns. *Cell Rep*. 2016;14(9):2142-2153. doi:10.1016/j.celrep.2016.02.013

40. Precup G, Vodnar DC. Gut Prevotella as a possible biomarker of diet and its eubiotic versus dysbiotic roles: a comprehensive literature review. *British Journal of Nutrition*. 2019;122(2):131-140. doi:10.1017/S0007114519000680

41. Vacca M, Celano G, Calabrese FM, Portincasa P, Gobbetti M, De Angelis M. The Controversial Role of Human Gut Lachnospiraceae. *Microorganisms*. 2020;8(4):573. doi:10.3390/microorganisms8040573

42. Ou J, Carbonero F, Zoetendal EG, et al. Diet, microbiota, and microbial metabolites in colon cancer risk in rural Africans and African

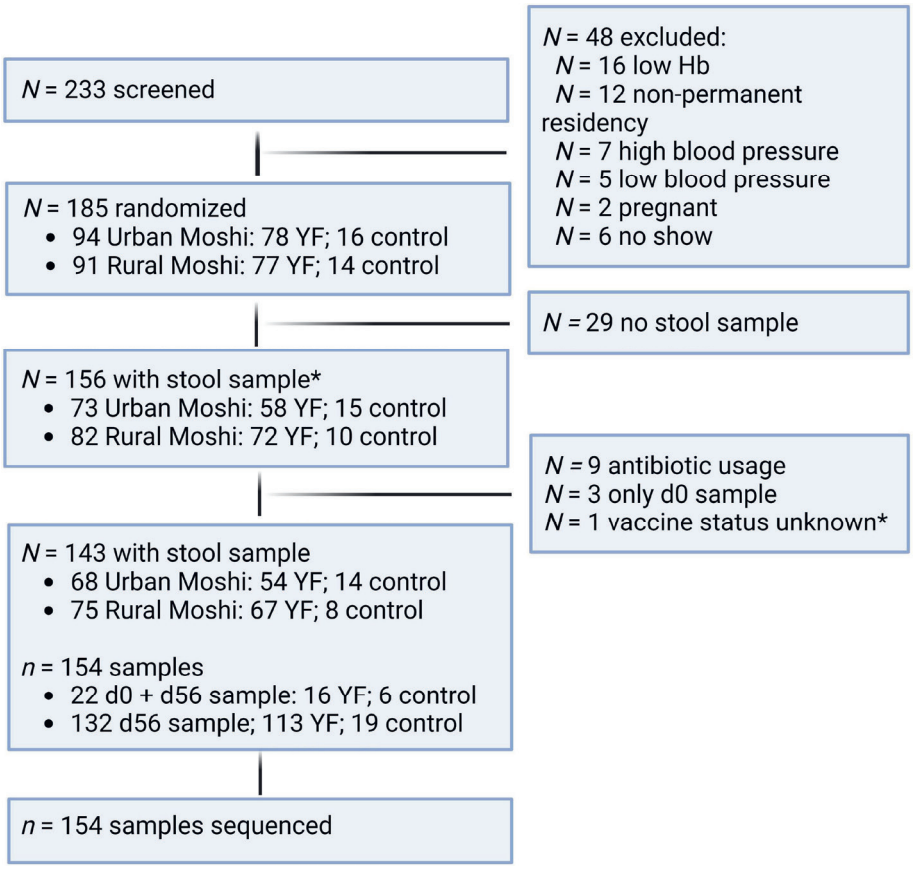
Americans. *Am J Clin Nutr*. 2013;98(1):111-120. doi:10.3945/ajcn.112.056689

43. Newman TM, Shively CA, Register TC, et al. Diet, obesity, and the gut microbiome as determinants modulating metabolic outcomes in a non-human primate model. *Microbiome*. 2021;9(1):100. doi:10.1186/s40168-021-01069-y
44. Sun J, Liao XP, D'Souza AW, et al. Environmental remodeling of human gut microbiota and antibiotic resistome in livestock farms. *Nat Commun*. 2020;11(1):1427. doi:10.1038/s41467-020-15222-y
45. Sudatip D, Mostacci N, Thamlikitkul V, Oppliger A, Hilty M. Influence of occupational exposure to pigs or chickens on human gut microbiota composition in Thailand. *One Health*. 2022;15:100463. doi:10.1016/j.onehlt.2022.100463
46. Nel Van Zyl K, Whitelaw AC, Hesseling AC, Seddon JA, Demers AM, Newton-Foot M. Association between clinical and environmental factors and the gut microbiota profiles in young South African children. *Sci Rep*. 2021;11(1):15895. doi:10.1038/s41598-021-95409-5
47. Rosa BA, Supali T, Gankpala L, et al. Differential human gut microbiome assemblages during soil-transmitted helminth infections in Indonesia and Liberia. *Microbiome*. 2018;6(1):33. doi:10.1186/s40168-018-0416-5
48. Mondot S, Poirier P, Abou-Bacar A, et al. Parasites and diet as main drivers of the Malagasy gut microbiome richness and function. *Sci Rep*. 2021;11(1):17630. doi:10.1038/s41598-021-96967-4
49. Walusimbi B, Lawson MAE, Nassuuna J, et al. The effects of helminth infections on the human gut microbiome: a systematic review and meta-analysis. *Frontiers in Microbiomes*. 2023;2. doi:10.3389/frmbi.2023.1174034
50. Schneeberger PHH, Gueuning M, Welsche S, et al. Different gut microbial communities correlate with efficacy of albendazole-ivermectin against soil-transmitted helminthiases. *Nat Commun*. 2022;13(1):1063. doi:10.1038/s41467-022-28658-1
51. van Dorst MMAR, Pyuza JJ, Nkurunungi G, et al. Immunological factors linked to geographical variation in vaccine responses. *Nat Rev Immunol*. 2024;24(4):250-263. doi:10.1038/s41577-023-00941-2
52. Muyanja E, Ssemaganda A, Ngauv P, et al. Immune activation alters cellular and humoral responses to yellow fever 17D vaccine. *Journal of Clinical Investigation*. 2014;124(7):3147-3158. doi:10.1172/JCI75429
53. Domingo C, Fraissinet J, Ansah PO, et al. Long-term immunity against yellow fever in children vaccinated during infancy: a longitudinal cohort study. *Lancet Infect Dis*. 2019;19(12):1363-1370. doi:10.1016/S1473-3099(19)30323-8
54. Jongo SA, Shekalaghe SA, Church LWP, et al. Safety, Immunogenicity, and Protective Efficacy against Controlled Human Malaria Infection of Plasmodium falciparum Sporozoite Vaccine in Tanzanian Adults. *Am J Trop Med Hyg*. 2018;99(2):338-349. doi:10.4269/ajtmh.17-1014
55. Querec TD, Akondy RS, Lee EK, et al. Systems biology approach predicts immunogenicity of the yellow fever vaccine in humans. *Nat Immunol*. 2009;10(1):116-125. doi:10.1038/ni.1688
56. Monath TP, Cetron MS, McCarthy K, et al. Yellow Fever 17D Vaccine Safety and

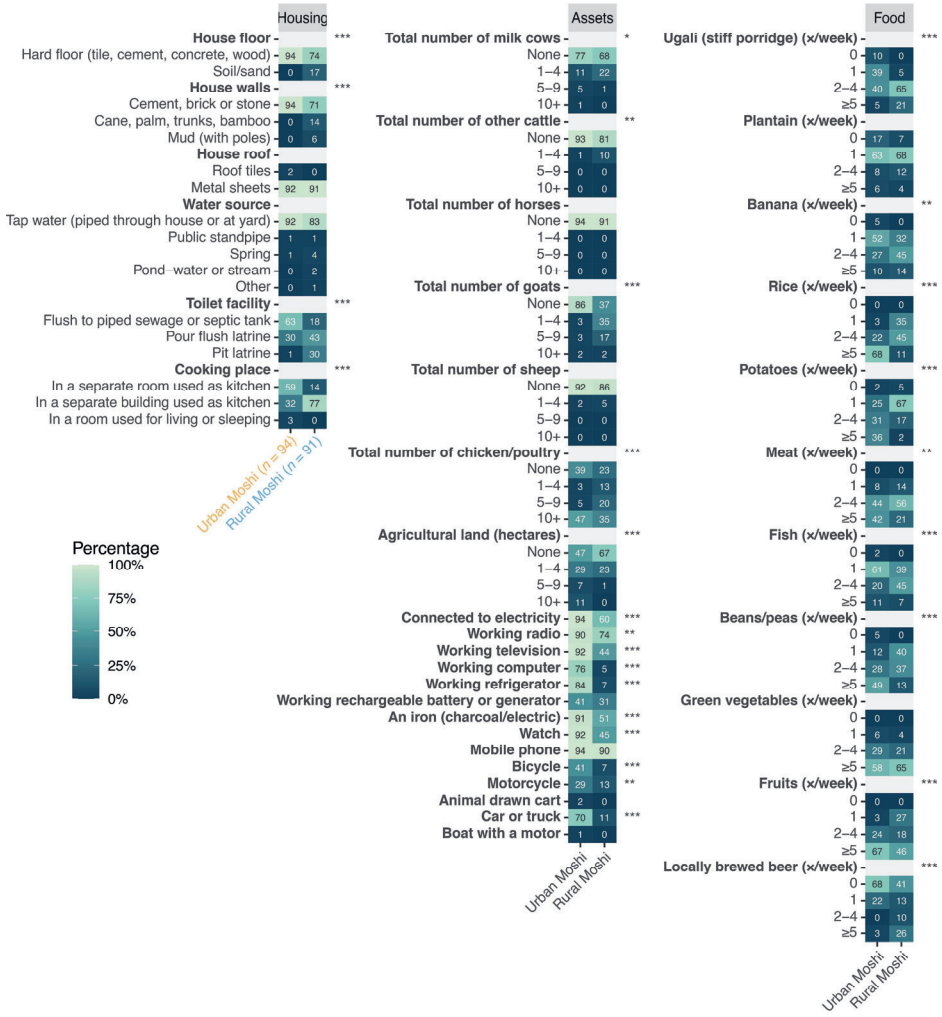
Immunogenicity in the Elderly. *Hum Vaccin*. 2005;1(5):207-214. doi:10.4161/hv.1.5.2221

57. Monath TP, Nichols R, Archambault WT, et al. Comparative safety and immunogenicity of two yellow fever 17D vaccines (ARILVAX and YF-VAX) in a phase III multicenter, double-blind clinical trial. *Am J Trop Med Hyg*. 2002;66(5):533-541. doi:10.4269/ajtmh.2002.66.533
58. A, Nkurunungi G, Zirimenya L, et al. Schistosome and malaria exposure and urban–rural differences in vaccine responses in Uganda: a causal mediation analysis using data from three linked randomised controlled trials. *Lancet Glob Health*. 2024;12(11):e1860-e1870. doi:10.1016/S2214-109X(24)00340-1
59. TNBS. Tanzania population and housing census- Tanzania National Bureau of statistics(TNBS). *https://www.nbs.go.tz/index.php/en/census-surveys/population-and-housing-census*. Published online 2022.
60. TDHS-MIS. Tanzania Demographic and Health Survey and Malaria Indicator Survey (TDHS-MIS) 2015-16. *https://dhsprogram.com/publications/publication-FR321-DHS-Final-Reports.cfm*. Published online 2016.
61. Cho SH, Raybuck AL, Stengel K, et al. Germinal centre hypoxia and regulation of antibody qualities by a hypoxia response system. *Nature*. 2016;537(7619):234-238. doi:10.1038/nature19334
62. Taylor CT, Scholz CC. The effect of HIF on metabolism and immunity. *Nat Rev Nephrol*. 2022;18(9):573-587. doi:10.1038/s41581-022-00587-8
63. Abbott RK, Thayer M, Labuda J, et al. Germinal Center Hypoxia Potentiates Immunoglobulin Class Switch Recombination. *The Journal of Immunology*. 2016;197(10):4014-4020. doi:10.4049/jimmunol.1601401
64. McGettrick AF, O'Neill LAJ. The Role of HIF in Immunity and Inflammation. *Cell Metab*. 2020;32(4):524-536. doi:10.1016/j.cmet.2020.08.002
65. Kim M, Qie Y, Park J, Kim CH. Gut Microbial Metabolites Fuel Host Antibody Responses. *Cell Host Microbe*. 2016;20(2):202-214. doi:10.1016/j.chom.2016.07.001
66. Martin M. Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet J*. 2011;17(1):10. doi:10.14806/ej.17.1.200
67. Edgar RC. Updating the 97% identity threshold for 16S ribosomal RNA OTUs. *Bioinformatics*. 2018;34(14):2371-2375. doi:10.1093/bioinformatics/bty113
68. Mallick H, Rahnavard A, McIver LJ, et al. Multivariable association discovery in population-scale meta-omics studies. *PLoS Comput Biol*. 2021;17(11):e1009442. doi:10.1371/journal.pcbi.1009442
69. Barnett D, Arts I, Penders J. microViz: an R package for microbiome data visualization and statistics. *J Open Source Softw*. 2021;6(63):3201. doi:10.21105/joss.03201

Supplementary Figure



Supplementary Figure 1 | Flowchart of volunteer recruitment, randomization and stool sample collection.
N = number of volunteers; *n* = number of samples. YF = Yellow Fever; d0 = day 0/baseline sample; d56 = day 56 sample. Low Hb, Hb < 8.5 g/dL; low blood pressure, blood pressure ≤90/60mmHg; high blood pressure, blood pressure ≥140/90mmHg. Non-permanent residency indicates individuals who upon further questioning had not lived at a given location for their whole life. *1 volunteer without recorded group/vaccine status at the moment of sample shipment.



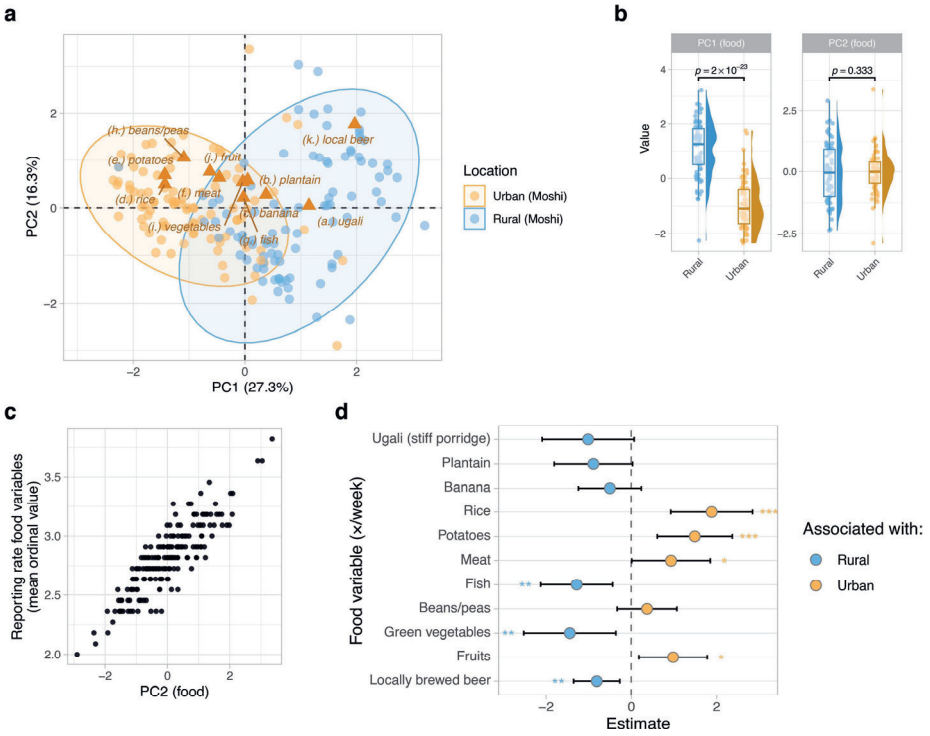
Supplementary Figure 2 | Heatmap visualizing lifestyle questionnaire data. *N* = 185 participants.

Values represent the number of participants. Colors indicate the percentage of the total. Comparisons between groups (rural/urban living) were performed using Fisher's exact or chi-squared tests. Asterisks denote statistical significance (NS, non-significant; *, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.001$, $p \leq 0.0001$). See Supplementary Table 2.

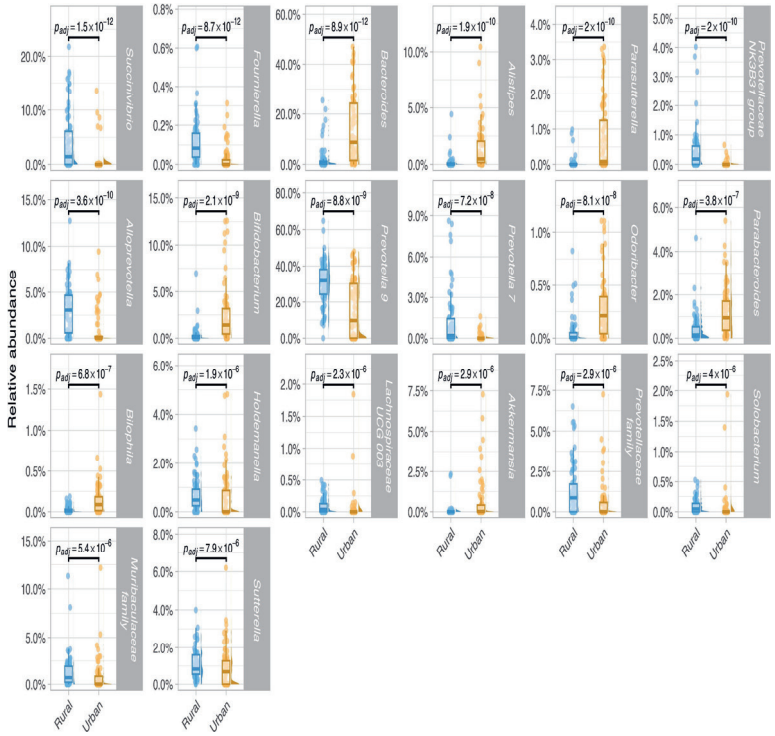
Supplementary Figure 3 | Multiple Correspondence Analysis (MCA) based on questionnaire data to generate lifestyle score.

a) MCA was applied to categorical questionnaire data (38 manually curated questions; 21 on assets, 11 on food and 6 on housing) ($N = 185$ individuals). Data points are colored based on location Ellipses reflect the data spread at a 95% confidence level. Density plots show the distribution of PC1 (lifestyle score) (x-axis) and PC2 (y-axis) score. b) Differences in lifestyle scores (PC1/PC2) between rural and urban living individuals. See legend **Figure 2a-b**. Statistical significance between groups was assessed using linear models with lifestyle score as outcome, adjusting for age and sex. c) Cumulative contributions (in percentage) of the variable categories by questionnaire data category

(i.e. housing, assets and food). d, f) Coordinates of each variable category most strongly contributing to PC1 (d) or PC2 (f) across dimensions 1 and 2. e, g) Contributions (in percentage) of variable categories to e) PC1 or lifestyle score or g) PC2 (based on lifestyle variables). Bars are colored based on PC1/PC2 scores as appropriate. h) Difference in lifestyle score across the overall cohort (N = 185) compared to the microbiota subcohort (N = 143), stratified by urban/rural living location. Statistical significance between groups was assessed using a linear model with lifestyle score as outcome.

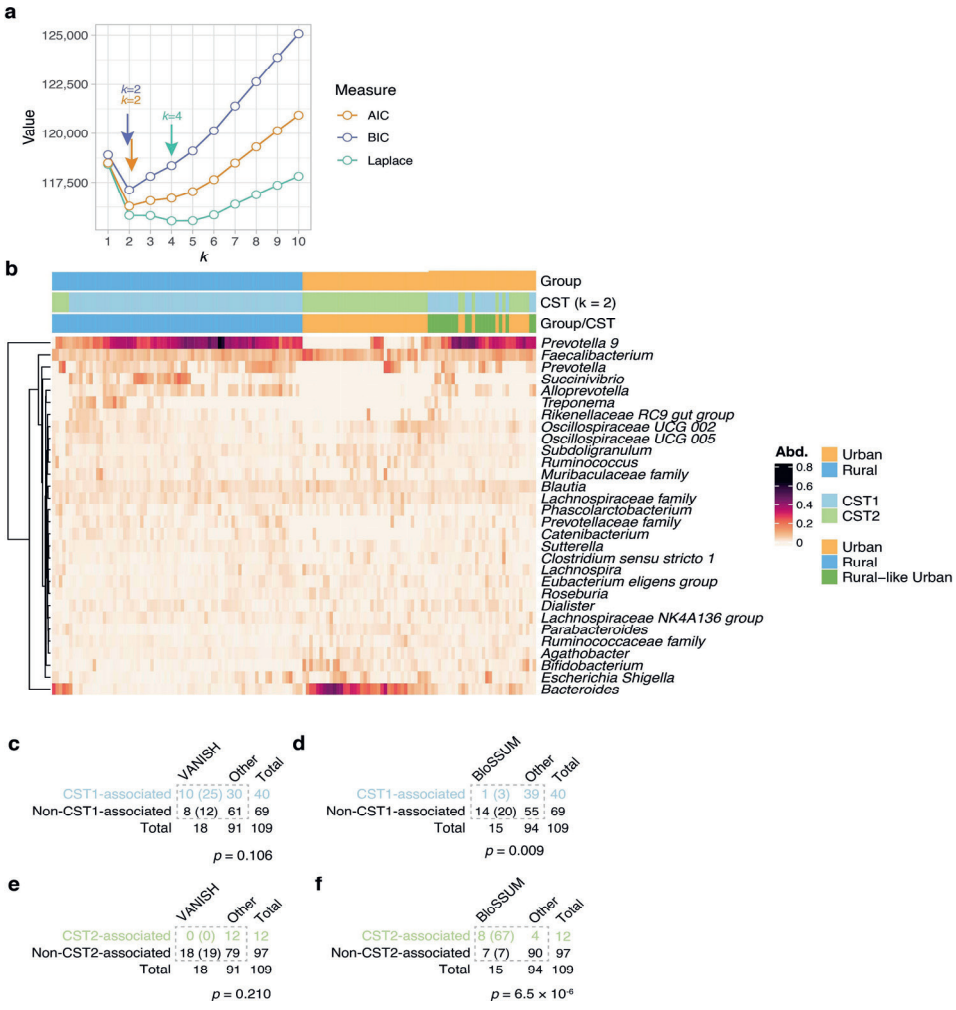


Supplementary Figure 4 | Principal component analysis (PCA) based on food variables to generate a food score. a) PCA was applied to 11 food-related variables (encoded as ordinal variables) (N = 185 individuals). Data points are colored based on location. Ellipses reflect the data spread at a 95% confidence level. b) Differences in food scores (PC1/PC2) between rural and urban living individuals. See legend Figure 2a-b. Statistical significance between groups was assessed using linear models with food score as outcome, adjusting for age and sex. c) Relation between PC2 food score and reporting rate of food variables (expressed as the mean ordinal value across 11 food variables for each individual). d) Association between food variables and living location (rural/urban; N = 185). Statistical significance was assessed using a logistic regression analysis with group as outcome and all food variables and sex as predictors. For each food variable, model estimates are depicted along the x-axis (colored points). Colors indicate the group with which a given food variable is positively associated with. Whiskers denote 95% confidence intervals (CIs; Wald-method). Asterisks denote statistical significance (*, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.001$).



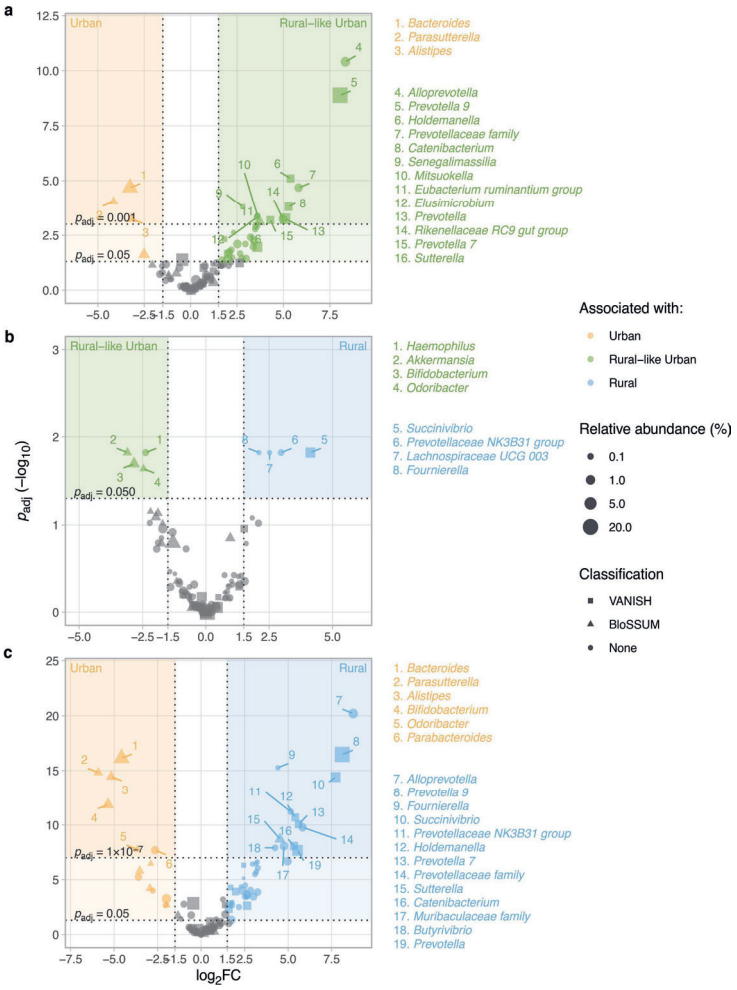
Supplementary Figure 5 | Boxplots of genera associated with rural/urban living location.

Only genera with $p_{adj} < 5 \times 10^{-5}$ and $\log_2$FC <-1.5 or >1.5 are shown. Statistical significance between groups (rural/urban living) was assessed using linear models with genus-level abundance as outcome, adjusting for vaccination status, age and sex. See legend **Figure 2a-b**.



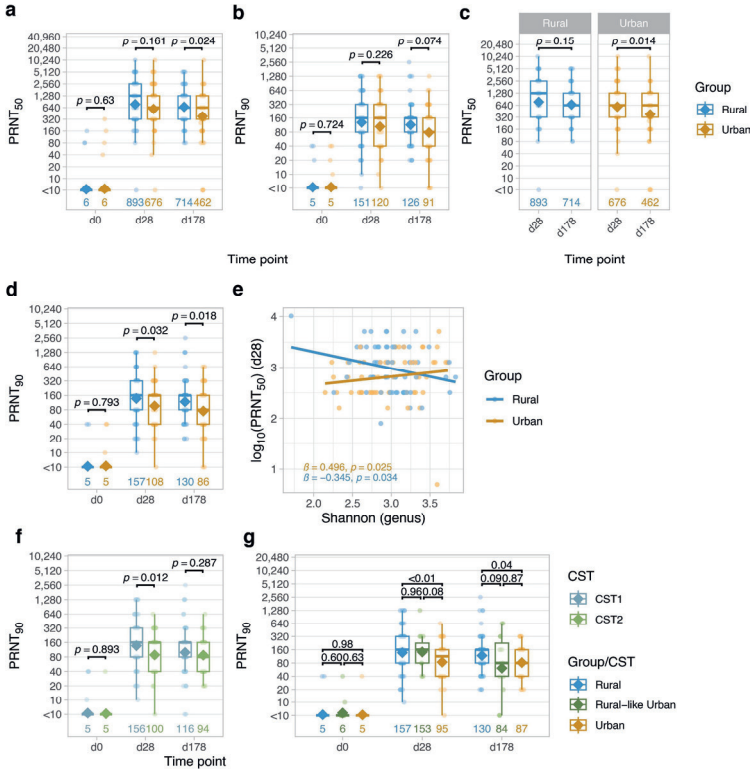
Supplementary Figure 6 | Microbiota profiles cluster in distinct Community State Types (CSTs) using Dirichlet-multinomial modelling (DMM).

a) DMM goodness-of-fit (AIC, BIC and Laplace) for k = 1-10 mixture components. For each goodness-of-fit measure, the optimal number of components is denoted using a colored arrow. b) Heatmap showing the relative abundance of the 30 most abundant genera for each sample. Samples are ordered based on living location; within living location samples are ordered according to Bray-Curtis dissimilarities (based on genus-level relative abundance) between samples. Taxa are ordered based on using hierarchical clustering (Euclidean distance) with optimal leaf ordering. Top horizontal bars indicate 1) living location, 2) CST allocation and 3) living location and CST membership combined. Urban living individuals belonging to CST1 were classified as rural-like urbanites since rural individuals almost exclusively clustered in CST1. For these comparisons, 'Urban' denotes urban individuals belonging to CST2. c-f 2 × 2 tables showing the number of VANISH (c, e) or BloSSUM taxa (d, f) across CST1- and CST2-associated taxa. Statistical significance was assessed using Fisher's Exact tests.



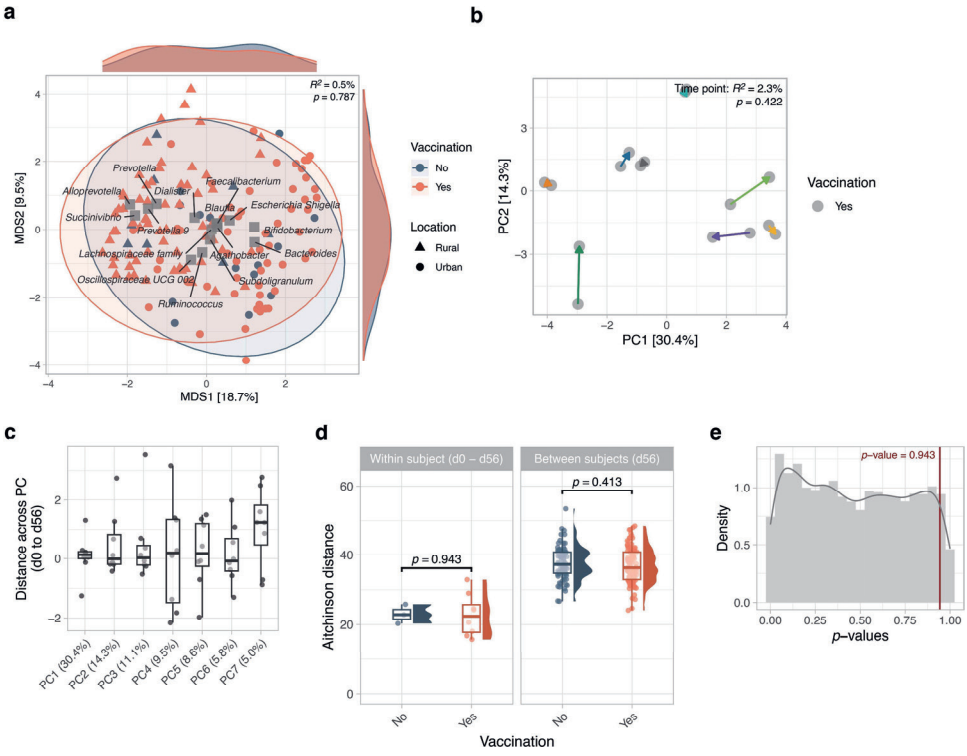
Supplementary Figure 7 | Differential abundance analyses urban/rural living individuals and rural-like urbanites.

a-c) Volcano plots visualizing genera enriched in a) urban (urban + CST2) living individuals compared to rural-like urbanites, b) rural-like urbanites compared to rural living individuals and c) urban (urban + CST2) compared to rural living individuals. Statistical significance between groups (rural/urban living/rural-like urbanites) was assessed using linear models with genus-level abundance as outcome (no adjustment for covariates). Model estimates and *p*-values for each contrast were extracted using the *emmeans* R-package. *P*-values were adjusted for multiple testing for each contrast. Genera with $p_{adj} < 0.05$ and $\log_2$-fold change (FC) <-1.5 or >1.5 are colored and genera with a $p_{adj} < 0.001$ (a), $p_{adj} < 0.05$ (b) or $p_{adj} < 1 \times 10^{-7}$ (c) and $\log_2$FC <-1.5 or >1.5 were annotated. The shape of the data points indicates whether these genera were previously considered to belong to VANISH (volatile and/or associated negatively with industrialized societies of humans) or BloSSUM (bloom or selected in societies of urbanization/modernization) taxa.



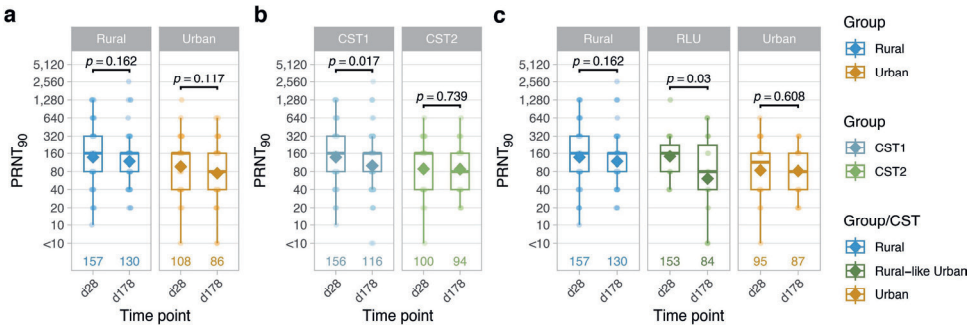
Supplementary Figure 8 | Yellow fever neutralizing antibody titer variation across living location and with microbiota profiles.

a-f) Boxplots (see legend Figure 2a-b) showing yellow neutralizing antibody titers (a; $PRNT_{50}$ and b-f; $PRNT_{90}$) in all vaccinated individuals ($N = 155$, a-c) and in those from whom a stool sample was collected ($N = 121$, d-f) at baseline (day 0, d0), 4 weeks post-vaccination (day 28, d28) and 6 months post-vaccination (day 178, d178). Boxplots show comparisons according to group (rural/urban living location; a-b), CST (e) and group/CST (i.e. rural living individuals, rural-like urbanites [CST1] and urban living individuals [CST2]) (f). c) Boxplots showing yellow neutralizing antibody titers ($PRNT_{50}$) at 4 weeks post-vaccination (day 28, d28) compared to 6 months post-vaccination (day 178, d178) for group (rural/urban living location). Diamond-shaped data points and color values at the bottom of the plot denote log_{10}-base geometric mean titers. Statistical analyses were performed using a generalized linear mixed effects model with log_{10}-transformed $PRNT_{50}$/$PRNT_{90}$-values as outcome, group, CST or group/CST, time point, the interaction between group, CST or group/CST and time point, age and sex as fixed effects and participant ID as random effects. Pairwise comparisons of estimated marginal means between groups were computed at each time point (a-b, d-f) or between day 28 and day 178 for each group (c). Values under the limit of detection (<10) were assumed to have a value of 5.



Supplementary Figure 9 | Association between YF-17D vaccination on gut microbiota profiles.

a) Principal component analysis (PCA) biplot using CLR-transformed genus-level microbiota features across day 56 samples. Data points, ellipses and side plots are colored based on vaccination status. See legend Figure 1C. R^2 and statistical significance of the association between vaccination status and the overall microbiota composition was assessed using PERMANOVA-test (1,000 permutations), while adjusting for living location, age and sex. b) Principal coordinate analysis of vaccinated volunteers with paired (day 0 – day 56) stool samples (N = 8, n = 16). Arrows indicate direction of 'movement' across PC1 and PC2 from day 0 to day 56. c) Boxplots (see legend Figure 2a-b) showing the distance from day 0 to day 56 across PC1-PC7. d) Within-subject distances (day 0 – day 56) between vaccinated (N = 8) and non-vaccinated individuals (N = 3) compared to between-subject distances (day 56). Distances between subjects were calculated for subjects with matching group (rural/urban living) and vaccination status (yes/no YF-17D vaccination). Up to 50 distances per stratum (rural – vaccine, urban – vaccine, rural – no vaccine and urban – no vaccine) were randomly selected (n = 28 distances were selected for rural – no vaccine). Statistical significance was assessed using a Student's t-test with Aitchison distance as dependent variable and vaccine status as independent variable. e) Permutation test based on subsampled between-subject distances between paired vaccinated and non-vaccinated subjects (n = 8 and n = 3, respectively). Histogram and line plot indicate the density distribution of p-values across 5,000 iterations. The 'true' observed within-subject p-value is shown in dark red.



Supplementary Figure 10 | Change in yellow fever neutralizing antibody titers at day 28 compared to day 178. a-c) Boxplots (see legend Figure 2a-b) showing yellow neutralizing antibody titers ($PRNT_{90}$) at 4 weeks post-vaccination (day 28, d28) compared to 6 months post-vaccination (day 178, d178) for rural compared to urban living individuals (a), CST1 compared to CST2 (b) and group/CST (i.e. rural living individuals, rural-like urbanites [CST1] [RLU] and urban living individuals [CST2]) (c) ($N = 121$). Diamond-shaped data points and color values at the bottom of the plot denote log_{10}-base geometric mean titers. Statistical analyses were performed using a generalized linear mixed effects model with log_{10}-transformed $PRNT_{90}$-values as outcome, group, CST or group/CST, time point, the interaction between group, CST or group/CST and time point, age and sex as fixed effects and participant ID as random effects. Pairwise comparisons of estimated marginal means between day 28 and day 178 were computed for each group. Values under the limit of detection (<10) were assumed to have a value of 5.

Supplementary tables

Supplementary Table 1 | Baseline characteristics of the study population (*N* = 185).

Variable	Overall, N = 185	Urban Moshi, *N* = 94	Rural Moshi, *N* = 91	p-value
Female sex	84 (45%)	50 (53%)	34 (37%)	0.031
Age	23.7 (21.2, 27.5)	23.6 (21.8, 26.1)	23.9 (20.3, 29.7)	0.813
Age categories				0.022
18-25	115 (62%)	66 (70%)	49 (54%)	
26-36	70 (38%)	28 (30%)	42 (46%)	
BMI	22.5 (20.0, 25.1)	23.6 (20.4, 26.4)	22.1 (19.9, 24.6)	0.076
BMI classification				0.228
<18.5	20 (11%)	9 (9.6%)	11 (12%)	
18.5-24.9	118 (64%)	55 (59%)	63 (69%)	
25.0-29.9	34 (18%)	22 (23%)	12 (13%)	
>30	13 (7.0%)	8 (8.5%)	5 (5.5%)	
Systolic blood pressure (mmHg)	116 (105, 124)	111 (102, 121)	120 (110, 129)	0.002
Diastolic blood pressure (mmHg)	71 (65, 78)	70 (64, 76)	72 (67, 79)	0.125
Hemoglobin level g/dl	15.00 (13.70, 16.20)	14.45 (12.70, 15.88)	15.40 (14.20, 16.50)	<0.001
Random blood sugar, mmol-1^^	5.20 (4.80, 5.80)	5.30 (4.90, 6.00)	4.90 (4.70, 5.45)	<0.001
Highest level of education				<0.001
Primary	52 (28%)	2 (2.1%)	50 (55%)	
Secondary	85 (46%)	47 (50%)	38 (42%)	
College	21 (11%)	20 (21%)	1 (1.1%)	
University	27 (15%)	25 (27%)	2 (2.2%)	
Helminth infection[a]	8 (4.3%)	0 (0%)	8 (8.8%)	0.003
Insurance status	80 (43%)	77 (82%)	3 (3.3%)	<0.001
Occupation				<0.001
Farming	39 (21%)	13 (14%)	26 (29%)	
Elementary occupation	59 (32%)	10 (11%)	49 (54%)	
Student	3 (1.6%)	3 (3.2%)	0 (0%)	
Employed/business owner	75 (41%)	64 (68%)	11 (12%)	
Not employed	1 (0.5%)	0 (0%)	1 (1.1%)	
Other	8 (4.3%)	4 (4.3%)	4 (4.4%)	
Received yellow fever vaccine	155 (84%)	78 (83%)	77 (85%)	0.763

N = 185 participants. Values represent number of participants (percentage of total) and median (interquartile range [IQR]) for categorical and continuous variables, respectively. Comparisons between locations were performed using Fisher's exact, chi-squared and Mann–Whitney U-test for categorical and continuous variables, respectively. [a]Stool was tested for helminths using the Kato-Katz method, testing for *Schistosoma mansoni*, *Ascaris lumbricoides*, hookworm and *Trichuris trichuria*. Additionally, urine was tested for *Schistosoma haematobium* and *Schistosoma mansoni* using the POC-CCA method.

Supplementary Table 2 | Descriptives of lifestyle score variables (*N* = 185).

Characteristic	Urban Moshi, *N* = 94	Rural Moshi, *N* = 91	p-value
House floor			<0.001
Hard floor (tile, cement, concrete, wood)	94 (100%)	74 (81%)	
Soil/sand	0 (0%)	17 (19%)	
House walls			<0.001
Cement, brick or stone	94 (100%)	71 (78%)	
Cane, palm, trunks, bamboo	0 (0%)	14 (15%)	
Mud (with poles)	0 (0%)	6 (6.6%)	
House roof			0.497
Roof tiles	2 (2.1%)	0 (0%)	
Metal sheets	92 (98%)	91 (100%)	
Water source			0.156
Tap water (piped through house or at yard)	92 (98%)	83 (91%)	
Public standpipe	1 (1.1%)	1 (1.1%)	
Spring	1 (1.1%)	4 (4.4%)	
Pond-water or stream	0 (0%)	2 (2.2%)	
Other	0 (0%)	1 (1.1%)	
Toilet facility			<0.001
Flush to piped sewage or septic tank	63 (67%)	18 (20%)	
Pour flush latrine	30 (32%)	43 (47%)	
Pit latrine	1 (1.1%)	30 (33%)	
Cooking place			<0.001
In a separate room used as kitchen	59 (63%)	14 (15%)	
In a separate building used as kitchen	32 (34%)	77 (85%)	
In a room used for living or sleeping	3 (3.2%)	0 (0%)	
Total number of milk cows			0.036
None	77 (82%)	68 (75%)	
1-4	11 (12%)	22 (24%)	
5-9	5 (5.3%)	1 (1.1%)	
10+	1 (1.1%)	0 (0%)	
Total number of other cattle			0.004
None	93 (99%)	81 (89%)	
1-4	1 (1.1%)	10 (11%)	
5-9	0 (0%)	0 (0%)	
10+	0 (0%)	0 (0%)	
Total number of horses			>0.999
None	94 (100%)	91 (100%)	
1-4	0 (0%)	0 (0%)	
5-9	0 (0%)	0 (0%)	

10+	0 (0%)	0 (0%)	
Total number of goats			<0.001
None	86 (91%)	37 (41%)	
1-4	3 (3.2%)	35 (38%)	
5-9	3 (3.2%)	17 (19%)	
10+	2 (2.1%)	2 (2.2%)	
Total number of sheep			0.273
None	92 (98%)	86 (95%)	
1-4	2 (2.1%)	5 (5.5%)	
5-9	0 (0%)	0 (0%)	
10+	0 (0%)	0 (0%)	
Total number of chicken/poultry			<0.001
None	39 (41%)	23 (25%)	
1-4	3 (3.2%)	13 (14%)	
5-9	5 (5.3%)	20 (22%)	
10+	47 (50%)	35 (38%)	
Agricultural land (hectares)			<0.001
None	47 (50%)	67 (74%)	
1-4	29 (31%)	23 (25%)	
5-9	7 (7.4%)	1 (1.1%)	
10+	11 (12%)	0 (0%)	
Connected to electricity	94 (100%)	60 (66%)	<0.001
Working radio	90 (96%)	74 (81%)	0.002
Working television	92 (98%)	44 (48%)	<0.001
Working computer	76 (81%)	5 (5.5%)	<0.001
Working refrigerator	84 (89%)	7 (7.7%)	<0.001
Working rechargeable battery or generator	41 (44%)	31 (34%)	0.183
An iron (charcoal/electric)	91 (97%)	51 (56%)	<0.001
Watch	92 (98%)	45 (49%)	<0.001
Mobile phone	94 (100%)	90 (99%)	0.492
Bicycle	41 (44%)	7 (7.7%)	<0.001
Motorcycle	29 (31%)	13 (14%)	0.007
Animal drawn cart	2 (2.1%)	0 (0%)	0.497
Car or truck	70 (74%)	11 (12%)	<0.001
Boat with a motor	1 (1.1%)	0 (0%)	>0.999
Ugali (maize stiff porridge) (×/week)			<0.001
0	10 (11%)	0 (0%)	
1	39 (41%)	5 (5.5%)	
2-4	40 (43%)	65 (71%)	
≥5	5 (5.3%)	21 (23%)	

Plantain (×/week)			0.134
0	17 (18%)	7 (7.7%)	
1	63 (67%)	68 (75%)	
2-4	8 (8.5%)	12 (13%)	
≥5	6 (6.4%)	4 (4.4%)	
Banana (×/week)			0.001
0	5 (5.3%)	0 (0%)	
1	52 (55%)	32 (35%)	
2-4	27 (29%)	45 (49%)	
≥5	10 (11%)	14 (15%)	
Rice (×/week)			<0.001
0	0 (0%)	0 (0%)	
1	3 (3.2%)	35 (38%)	
2-4	22 (24%)	45 (49%)	
≥5	68 (73%)	11 (12%)	
Missing	1	0	
Potatoes (×/week)			<0.001
0	2 (2.1%)	5 (5.5%)	
1	25 (27%)	67 (74%)	
2-4	31 (33%)	17 (19%)	
≥5	36 (38%)	2 (2.2%)	
Meat (×/week)			0.007
0	0 (0%)	0 (0%)	
1	8 (8.5%)	14 (15%)	
2-4	44 (47%)	56 (62%)	
≥5	42 (45%)	21 (23%)	
Fish (×/week)			<0.001
0	2 (2.1%)	0 (0%)	
1	61 (65%)	39 (43%)	
2-4	20 (21%)	45 (49%)	
≥5	11 (12%)	7 (7.7%)	
Beans/peas (×/week)			<0.001
0	5 (5.3%)	0 (0%)	
1	12 (13%)	40 (44%)	
2-4	28 (30%)	37 (41%)	
≥5	49 (52%)	13 (14%)	
Missing	0	1	
Green vegetables (×/week)			0.347
0	0 (0%)	0 (0%)	
1	6 (6.5%)	4 (4.4%)	

2-4	29 (31%)	21 (23%)	
≥5	58 (62%)	65 (72%)	
Missing	1	1	
Fruits (×/week)			<0.001
0	0 (0%)	0 (0%)	
1	3 (3.2%)	27 (30%)	
2-4	24 (26%)	18 (20%)	
≥5	67 (71%)	46 (51%)	
Locally brewed beer (×/week)			<0.001
0	68 (73%)	41 (46%)	
1	22 (24%)	13 (14%)	
2-4	0 (0%)	10 (11%)	
≥5	3 (3.2%)	26 (29%)	
Missing	1	1	

N = 185 participants. Values represent number of participants (percentage of total). Comparisons between locations were performed using Fisher's exact or chi-squared tests. All variables (*n* = 38 variables), after mode imputation, were used to construct the lifestyle score. See **Supplementary Figure 2**.

Supplementary Table 3 | Baseline characteristics of the rural-like urbanites compared to other urban living individuals (*N* = 69).

Variable	Overall, *N* = 69	Rural-like Urban, *N* = 21	Urban, *N* = 48	p-value
Female sex	36 (52%)	6 (29%)	30 (63%)	0.009
Age	23.0 (21.2, 24.6)	23.1 (21.8, 24.6)	22.9 (21.2, 24.5)	0.676
Age categories				>0.999
18-25	55 (80%)	17 (81%)	38 (79%)	
26-36	14 (20%)	4 (19%)	10 (21%)	
BMI	23.1 (20.4, 26.4)	23.8 (20.7, 26.3)	22.8 (19.8, 26.5)	0.825
BMI classification				0.575
<18.5	6 (8.7%)	3 (14%)	3 (6.3%)	
18.5-24.9	42 (61%)	11 (52%)	31 (65%)	
25.0-29.9	16 (23%)	6 (29%)	10 (21%)	
>30	5 (7.2%)	1 (4.8%)	4 (8.3%)	
Systolic blood pressure (mmHg)	110 (102, 120)	110 (107, 120)	111 (100, 121)	0.481
Diastolic blood pressure (mmHg)	70 (63, 76)	70 (60, 77)	70 (64, 75)	0.958
Hemoglobin level g/dl	14.30 (12.60, 15.60)	14.90 (14.20, 15.80)	13.65 (12.43, 15.28)	0.022
Random blood sugar, mmol-1^^	5.30 (4.90, 6.00)	5.30 (5.10, 5.60)	5.30 (4.90, 6.00)	0.759
Highest level of education				0.593
Primary	2 (2.9%)	1 (4.8%)	1 (2.1%)	
Secondary	38 (55%)	10 (48%)	28 (58%)	

College	14 (20%)	4 (19%)	10 (21%)	
University	15 (22%)	6 (29%)	9 (19%)	
Insurance status	60 (87%)	18 (86%)	42 (88%)	>0.999
Occupation				0.690
Farming	10 (14%)	2 (9.5%)	8 (17%)	
Elementary occupation	7 (10%)	2 (9.5%)	5 (10%)	
Employed/business owner	49 (71%)	17 (81%)	32 (67%)	
Other	3 (4.3%)	0 (0%)	3 (6.3%)	
Received yellow fever vaccine	55 (80%)	15 (71%)	40 (83%)	0.332

N = 69 urban living participants. Values represent number of participants (percentage of total) and median (interquartile range [IQR]) for categorical and continuous variables, respectively. Comparisons between locations were performed using Fisher's exact, chi-squared and Mann–Whitney U-test for categorical and continuous variables, respectively.

Chapter 7

Summary, general discussion and future perspective

Summary

In Chapter 1, we introduced the factors associated with variation in the immune system and the differences in immune responses to vaccines. Additionally, we outlined the main objective of this thesis, the study designs, the geographical areas where the studies were conducted, and the study populations recruited. An outline of the subsequent chapters of the thesis was also provided.

In Chapter 2, we reviewed the significant challenge that vaccine hypo-responsiveness to certain vaccines poses to global health, particularly due to the variability in vaccine efficacy across different populations and geographical regions. This is especially pronounced in low- and middle-income countries, where vaccines for diseases such as malaria, tuberculosis (TB) and rotavirus often demonstrate reduced immunogenicity and, for some even effectiveness, compared to those in high-income regions. Our review identified several contributing factors to vaccine hypo-responsiveness, including environmental and lifestyle factors such as exposure to microorganisms and parasites (such as HIV, CMV, malaria, helminths, and environmental mycobacteria), variations in the microbiome (such as phage diversity, commensal bacteria), and the presence of pro-inflammatory and anti-inflammatory metabolites (for example, flavones).

We further explored the potential immunological mechanisms underlying poor responses to vaccines including pre-existing immunity or cross-reactive antigens, persistent immune activation, immune exhaustion, immune senescence, and alterations in tissue micro-environments, such as in lymph nodes, and skewed immune responses. In addition, we reviewed potential strategies to reverse or enhance vaccine responses. These strategies include the change in the use of adjuvants and adjustments to vaccine regimens, reduction of inflammation, application of checkpoint and MAPK inhibitors, modifications of lymphoid stromal cells, and the use of monoclonal antibodies targeting TH2 cytokines and Treg-cells. We concluded that the application of advanced omics technologies and further exploration of the roles of immune metabolism and local microenvironments could provide deeper insights into the mechanisms behind vaccine hypo-responsiveness. Such understanding is crucial for the development of tailored vaccination strategies that can effectively overcome these barriers.

As helminths seem to be important immune modulators, their prevalence in different geographical areas might need to be assessed when studying vaccine responses. To achieve this, sensitive and specific diagnostic tools are needed.

In chapter 3, we investigated the current prevalence of schistosomiasis among school-aged children in Mwanga district, Tanzania, after nearly two decades of Mass Drug Administration (MDA) with praziquantel. While urine microscopy remains the conventional gold standard for diagnosing urinary schistosomiasis in endemic settings, its sensitivity is limited. Therefore, we employed the Up-Converting Particle Lateral Flow Circulating Anodic Antigen (UCP-LF CAA) test, known for its high sensitivity in detecting active infections but needs a laboratory-based reading machine. We also explored the potential of using the Point-of-Care Circulating Cathodic Antigen (POC-CCA) test and the micro-haematuria dipstick as combined diagnostic tools in comparison to the UCP-LF CAA test.

Our findings indicated a moderate prevalence of schistosomiasis of 20.3% based on the UCP-LF CAA test, which provided a more accurate reflection of the current disease burden than the combined POC-CCA and micro-haematuria tests. The latter showed higher prevalence rates, but the poor agreement with the UCP-LF CAA test, questions the reliability of the POC-CCA. The POC-CCA test appears to show variability due to factors such as production batch differences, variability in test sensitivity, and the subjective nature of the interpretation of the results.

Our study underscores the persistent transmission of schistosomiasis in the region despite long-term MDA efforts. It also emphasizes the need for improved diagnostic tools that can be applied directly as a point-of-care test in the field without the need for any apparatus. Such a test would need to be integrated into control strategies that consider local transmission dynamics and socio-environmental factors. These advancements are crucial for achieving more effective disease management and moving closer to the goal of schistosomiasis elimination in endemic regions.

In Chapter 4, we investigated the association between lifestyle factors and cellular immune profiles in healthy Tanzanian adults. The lifestyle score was developed based on household assets, housing conditions, and dietary history. First, using rural-urban locations: we found significant differences in immune cell frequencies between rural and urban participants. Rural

participants exhibited higher frequencies of Th2-cells, atypical memory B-cells, and various subsets of CD4+ T effector memory (Tem)-cells, including those expressing markers like CD38, HLA-DR, PD-1, KLRG-1 and CTLA-4. Indicating a more activated and regulatory immune state.

Importantly, the lifestyle score confirmed five immune cell clusters previously identified by geographical location alone. These included clusters of Th2-cells, CD4+ Tem-cells, atypical memory B-cells, and CD8+ Tem-cells. These clusters were predominantly associated with rural living and a lower lifestyle score. Additionally, the lifestyle score identified eight unique immune cell clusters that were not detected when considering geographical location alone. Lower lifestyle scores were linked to higher frequencies of plasmablasts, regulatory T-cells, and NK-cells, while higher lifestyle scores, typically associated with urban living, correlated with increased frequencies of naïve CD8+ T-cells and CD8+ Tem-cells expressing markers like CD161 and KLRG-1. We concluded that lifestyle factors significantly shape cellular immune profiles beyond the influence of geographical location alone. This enhanced understanding of lifestyle-driven immune variation is crucial for improving vaccine responses and managing immune-related diseases, particularly in diverse and low- and middle-income populations.

In Chapter 5, Following the findings of immunological differences across geographical areas, we were interested in the impact of these differences on vaccine responses. To this end, we compared yellow fever vaccine immunogenicity in rural and urban Moshi. Immunogenicity was measured using a clinically important neutralization assay, which allowed us to identify protected and non-protected subjects. In addition, we measured antibodies against non-structural protein 1 (NS1) using ELISA, providing a robust continuous dataset representing vaccine immunogenicity.

In Chapter 6, we investigated the association of gut microbiota composition in rural- and urban-living Tanzanian adults concerning the yellow fever vaccine antibody response. We found significant differences in the gut microbiota composition between individuals living in rural and urban areas. In rural Tanzanians, the gut microbiota was more diverse, with a higher prevalence of genera such as *Prevotella*, *Succinivibrio*, and *Treponema*. Rural diets in this study were characterized by a higher intake of traditional foods like ugali (a stiff porridge made from maize), vegetables, and locally brewed beer, which altogether represent plant-based diets

rich in fibres and complex carbohydrates. In contrast, urban Tanzanians displayed a gut microbiota dominated by *Bacteroides*, *Parabacteroides*, and members of the *Enterobacteriaceae* family. These bacterial genera are typically associated with diets high in processed foods and refined carbohydrates, food consumed by most in urban areas for example rice and potatoes in the form of chips. Interestingly, we identified a subset of urban individuals with a microbiota composition resembling that of rural inhabitants, termed "rural-like urban" individuals. These individuals consumed more beans and peas, foods high in proteins and dietary fibres while consuming less rice and ugali, aligning their microbiota profiles more closely with those of rural individuals.

Urban-living individuals were significantly enriched for BloSSUM taxa (bloom or selected in societies of urbanization/modernization), while rural-living individuals lacked these genera, with no significant enrichment of VANISH taxa (volatile and/or associated negatively with industrialized societies of humans) in rural-associated genera. Regarding yellow fever vaccine antibody titer, we found that rural individuals generally exhibited higher yellow fever neutralizing antibody titers compared to their urban counterparts. Notably, within the urban population, those with a rural-like microbiota profile showed higher initial antibody titers but also experienced stronger waning over time, like the rural group. The findings indicate that the gut microbiota, influenced partly by diet, might have the ability to modulate vaccine responses. The study findings emphasize the potential for microbiota-targeted interventions, such as dietary modifications, to improve vaccine efficacy, particularly in populations undergoing rapid urbanization and dietary transitions.

In Chapter 7, we discussed the main findings of this thesis, focusing on a select few factors associated with variations in the immune system and differences in immune responses to vaccines. Additionally, we explored future perspectives and concluded with the main conclusions of this thesis.

General discussion

The role of diagnostic tools in understanding the immune system

In **Chapter 3**, of this thesis, we investigated the current prevalence of schistosomiasis among school-aged children in Mwanga District, Tanzania, following nearly two decades of Mass Drug Administration (MDA) with praziquantel. Utilizing the highly sensitive and specific Up-Converting Particle Lateral Flow Circulating Anodic Antigen (UCP-LF CAA) test[1], we found a schistosomiasis prevalence of 20.3%. This contrasts with a 65.3% prevalence detected when combining the Point-of-Care Circulating Cathodic Antigen (POC-CCA) test and microhematuria dipstick. These results suggest that schistosomiasis remains prevalent in the area, indicating ongoing transmission. Furthermore, they underscore the importance of the sensitivity and specificity of diagnostic tools in accurately assessing disease prevalence. The lower prevalence detected by the ultra-sensitive CAA test[1] likely reflects a true decline from the 51.8% average prevalence recorded in 2005 using the Kato-Katz egg method, which may have underestimated the true burden of the disease due to its low sensitivity[2]. Indeed relying on a single Kato-Katz test can lead to an underestimation of prevalence by as much as 50%[3]. This decline could be attributed to the ongoing mass drug administration (MDA) program, which administers praziquantel annually to primary school-aged children.

To explore immunological differences between rural and urban settings, we extended our study to adults from the same rural areas, as discussed in Chapter 4. In this adult population from Mwanga, the prevalence of schistosomiasis was 4%, as determined by the Kato-Katz test and POC-CCA. Given that adults are typically not included in the MDA programs, and their primary economic activities such as agriculture or fishing involve water contact, the most plausible contribution to their lower infection rates may be age-related acquired immunity[4]. Additionally, the lower sensitivity of the Kato-Katz test, especially in low-endemic areas where egg output is low, might have underestimated the true prevalence[5]. Other factors contributing to the observed low prevalence may include environmental changes, preventive strategies, and increased self-deworming practices. Despite the low prevalence detected by Kato-Katz and POC-CCA, we observed high frequencies of Th2-cells in the adult population, suggesting that these individuals might still harbour schistosomiasis infections that were not detected by Kato-Katz, or that the elevated Th2 response could result from previous/historical infections or other lifestyle factors.

These findings emphasize the critical role of high-quality diagnostic tools in accurately estimating schistosomiasis prevalence and understanding the immunological variations associated with the infection. Schistosomiasis-infected individuals typically exhibit elevated Th2 responses, which decrease following treatment with anti-helminthic drugs, alongside other immunological changes, including reductions in T regulatory cells [6, 7]. In studies such as Human Controlled Infection Models (CHIMs) for schistosomiasis, the use of CAA tests has proven invaluable in confirming infection and correlating antigen levels with specific immune responses, thus linking immunological changes to infection dynamics[8].

Conversely, poor-quality diagnostic tools, whether due to variability in sensitivity, specificity, or technical errors, can lead to missed infections or overestimation of prevalence. This can obscure our understanding of the factors driving immune variation and hinder efforts to address the impacts on vaccine response variation. Given the lower sensitivity of the Kato-Katz test, particularly in low transmission areas, CAA tests provide a more reliable option for monitoring schistosomiasis and understanding its immunopathology. Regarding the effect on vaccine responses, future vaccine studies in areas endemic for helminth infections could investigate whether current infection is associated with variation in vaccine responses. This could be done by designing treatment studies in healthy adults, like the study conducted in Uganda, where pregnant women and their children were dewormed, and their cytokine levels were measured[9].

The association between lifestyle factors and cellular immune profiles

In **Chapter 4**, we examined the relationship between lifestyle factors and cellular immune profiles in healthy Tanzanian adults. Our study revealed significant differences in immune cell frequencies between rural and urban participants. Individuals from rural areas exhibited higher frequencies of Th2-cells, memory CD4 T-cells, atypical memory B-cells, and increased activation of these cells compared to their urban counterparts. Conversely, urban individuals showed higher frequencies of naïve gamma delta T-cells, CD4+ T central memory-cells, and $CXR3^{+}$, $CD8^{+}$ T-cells. These differences are likely due to current or past environmental exposures, particularly to infections such as parasitic diseases. Individuals in rural settings are more at risk of acquiring such infections due to socio-economic activities like agriculture, fishing, and recreational activities (swimming), but also limited resources that mean exposure to contaminated water and poor hygiene, all drivers of an activated immune system.

Our findings align with other studies, such as those comparing rural and urban populations in Senegal and Indonesia[6, 10]. In these studies, rural populations, particularly in Senegal, had higher frequencies of Th2-cells, pro-inflammatory cytokines, memory CD4+ T-cells, and memory B-cells, indicating a heightened immune response[10]. Similarly, rural individuals in Indonesia exhibited an activated immune status, characterized by higher frequencies of Th2 and regulatory T-cells, mirroring some of our findings[6]. While previous studies have linked these activated immune states to helminth infections, shown by a decrease in specific Th2 and regulatory T-cells post-treatment[6, 7], the low prevalence of helminth infections in our study prompted us to explore other factors contributing to the observed immune activation.

To further investigate the basis of these immunological differences, we assessed the relationship between lifestyle scores and immune profiles. The lifestyle score was developed based on household assets, housing conditions, and dietary history, capturing data on housing (e.g., type of floor, wall materials, electricity connection, toilet facilities), asset ownership (e.g., bicycles, cars, radios, TVs, refrigerators, computers), and dietary habits (e.g., types of food consumed weekly). Using this lifestyle score, we were able to detect and confirm clusters observed using the rural-urban scale. Additionally, this approach highlighted immune cell clusters uniquely associated with lifestyle factors, which were not identified using the rural-urban dichotomy. The question is why these clusters were not observed when considering the rural-urban dichotomy. The identification of additional and unique cell clusters not observed with the rural-urban gradient indicates that lifestyle factors, such as those used in our study, offer a more granular understanding of immune profiles. For example, socio-economic status, as reflected in asset ownership, has the potential to capture more subtle influences, such as the impact of low socio-economic status on stress levels, nutrition, and environmental exposures, which may have contributed to the additional clusters we identified. Furthermore, dietary history, which can influence the immune system, likely plays a role in additional clusters.

We found that lower lifestyle scores were associated with higher frequencies of plasmablasts, regulatory T-cells, and NK-cells. In contrast, higher lifestyle scores, typically linked to urban living, correlated with increased frequencies of naïve CD8+ T-cells and CD8+ Tem-cells expressing markers such as CD161 and KLRG-1. These may include MAIT-cells, which are commonly observed in urban environments; however, we were unable to confirm this due to the absence of TCRva72.

First, this indicates that individuals with lower lifestyle scores have an activated immune state, given the very low prevalence of helminth infections, other factors, though not measured in this thesis, such as viral infections like CMV[11], the presence of ectoparasites (e.g., ticks)[12], fungal exposure in living environments and food[13, 14], nutrition[15, 16], and animal contact, may partly explain the observed immune activation. Indeed, in our study housing conditions, asset ownership (socio-economic status), diet, and nutritional history differed significantly between rural and urban individuals. Upon further consideration, individuals with a higher lifestyle score tend to exhibit a less activated immune state, characterized by a greater number of naïve T-cells. However, these individuals also possess an increased number of CD8+ Tem-cells expressing CD161 and KLRG1. As previously mentioned, the possibility that these are MAIT-cells cannot be ruled out. The CD161 marker is associated with enhanced cytotoxic activities and shows high expression of IFN-γ upon activation, which is observed in various conditions such as viral infections and inflammatory states[17]. Conversely, KLRG1 serves as an inhibitory marker capable of downregulating immune activation by suppressing cytotoxicity, inhibiting cytokine production, or through other mechanisms[18-20]. Given that urban individuals are generally less exposed to pathogens, aside from common seasonal viral infections, this expression pattern may reflect a physiological mechanism aimed at controlling inflammatory conditions, such as autoimmune diseases or latent infections such as CMV or EBV. Urban living is associated with a higher risk of inflammatory conditions, including allergies and autoimmune diseases[21, 22] suggesting that the body is in a constant state of regulatory adjustment to mitigate these risks.

Vaccine hypo-responsiveness in a state context

In **Chapter 5**, we investigated whether vaccine responses differed between rural and urban individuals. Contrary to the initial hypothesis, which proposed that urban individuals would exhibit a stronger immune response due to potentially lower exposure to pathogens, better healthcare access, healthier diets, and higher socioeconomic status, our study revealed that rural individuals generally exhibited higher yellow fever-neutralizing antibody levels compared to their urban counterparts. Given that rural populations are often expected to have poorer responses to vaccines, these findings suggest that the variation in vaccine response is context-dependent, and influenced by individual characteristics, the type of vaccine, and other factors. In this case, rural individuals have shown better responses to vaccines, and possibly

better protection, compared to those in urban settings. This challenges the assumption of generalized vaccine hypo-responsiveness and highlights the complexity of immune responses to vaccines, calling for a tailored approach in vaccination strategies.

Contradictory findings such as these have also been reported in other studies on yellow fever vaccines. For example, a large trial involving individuals from North America and the United Kingdom found no significant difference in yellow fever immunogenicity between young and elderly populations[23-25]. Similarly, our findings contrast with several previous studies, such as those comparing children vaccinated with yellow fever in rural Ghana and urban Mali[26], and in Schistosoma-endemic areas of Uganda versus urban populations, where lower antibody titers were observed in rural settings[27]. Additionally, a study comparing yellow fever-vaccinated individuals between Uganda and Switzerland showed that Ugandan adults had lower antibody levels than those in Switzerland[28].

The differences between our findings and those from other locations might be explained by varying rates of pathogen exposure. Pathogen exposure is known to affect the immune system and able to reduce vaccine efficacy[29]. In our study, rural individuals had no history of malaria exposure and tested negative for malaria; there was also a very low prevalence of parasitic infections, and we did not find for example schistosomiasis, a pathogen notorious for its ability to modulate the immune system[30]. The difference between our study and that conducted in Uganda might point towards a crucial role in the ability of parasitic infections to profoundly affect the immune system and thereby responses to vaccines. Moreover, possibly related to this, is that an optimal immunological age for immune priming is reached in our young adults from rural areas, compared to the younger individuals, and adolescents studied in Uganda where a stable level of immune activity enables a better vaccine response. The differences in exposure to cross reactive pathogens might also be different between Tanzania and Uganda. A direct comparison of the baseline immunological profiles of the Ugandan study with ours might be able to shed light on the differences in YF vaccine responses across rural and urban areas.

Another possible reason for the observed differences is the nature of the gut microbiome. Rural individuals typically have a more diverse gut microbiome, which is becoming recognized as a key factor influencing immune function and vaccine response. Unlike most other LMICs, the gut microbiome of our study population might be more balanced due to better nutrition and a

lower prevalence of pathogens that can cause enteropathy and affect vaccine response[31]. Rural living individuals have nutrient-rich diets due to the geographical location on the slopes of Mount Kilimanjaro, a volcanic mountain with fertile soil and abundant water sources for agriculture, supporting a variety of high-quality foods, including maize, bananas, beans, fruits (avocado, mangoes) and green vegetables[32]. This difference in dietary history may have contributed to the enhanced vaccine response observed. Other factors, such as better socioeconomic status resulting from coffee sales and better access to healthcare services compared to most other rural settings in African countries, can also influence the findings.
An interesting baseline difference between rural and urban individuals that might have influenced the higher antibody levels was the haemoglobin level. Rural individuals had significantly higher haemoglobin levels compared to urban individuals, which contrasts with several findings in Africa, where lower haemoglobin levels have been reported in rural compared to urban populations[33, 34], within the rural population, individuals with higher haemoglobin levels were found to have higher antibody titers. This suggests that haemoglobin levels might be a contributing factor to the variation in vaccine response Different factors, such as nutrient type and high altitude, could explain this. Indeed rural individuals live in altitudes ranging from 1800-2145 meters above sea level compared to 700-950 meters above sea level in urban settings[35]. High altitude, which is associated with hypoxic conditions, can increase the transcription factor hypoxia-inducible factor (HIF). This, in turn, induces metabolic and phenotypic changes in B-cells, boosts B-cell differentiation[36-39], and enhances CD4+ T-cell function[40]. The enhanced T-cell function promotes the production of cytokines, which are important for antibody production and class switching by B-cells. Indeed, studies in animals have shown that hypoxia-inducible factors in CD4+ T-cells are crucial for effective humoral immunity, as they enhance glycolysis, and cytokine production, and regulate T-cell subsets[40]. It would be interesting to measure HIF levels in the blood samples collected to ascertain if there is a statistically significant difference between rural and urban populations.

Our findings exemplify the complexity of findings from population studies. For instance, studies conducted in the same geographical areas as ours, showed a significant upregulation of inflammatory genes, accompanied by higher *ex-vivo* cytokine levels among urban compared with rural Tanzanians [41]. A genomic study comparing Tanzanians and Europeans revealed that Tanzanian populations have an enrichment of the interferon pathway compared to Europeans[42].

In **Chapter 2** of this thesis, we extensively reviewed those inflammatory pathways, particularly those involving NF-κB and IRF7, that play a crucial role in predicting vaccine responses[43]. In studies done mostly in Western populations, it is known that type 1 interferon pathways are important and commonly upregulated shortly after vaccination, especially live attenuated viral vaccines and during infections[44, 45].

Given these observations, it would be expected that urban individuals would have higher antibody titers compared to rural individuals; however, this was not the case in our study. Several factors could explain this, including environmental such as living altitude, and microbial exposure. Interestingly, despite the observed enrichment of inflammatory pathways among Tanzanians, studies have also found that these pathways are enriched with anti-inflammatory cytokines like IL-10, highlighting that pro-inflammatory responses are integrated with anti-inflammatory regulation[42]. This raises questions about whether immunological studies conducted in Western contexts can be equally applicable to individuals in low- and middle-income countries. Taken together, the higher antibody titers among rural individuals suggest that vaccine response is a dynamic phenomenon, and no single hypothesis so far could fully explain the observed differences. Furthermore, the status of hypo-responsiveness in certain demographics, such as rural populations, the elderly, or infants, may not be universally applicable. Therefore, we should approach this in a more context-dependent manner, considering environmental factors, geographical location, characteristics of the individuals studied, vaccine types and antigen specificities. This approach will help in explaining findings and support context-based decision-making based on actual results.

Gut microbiome composition

In **Chapter 5**, we explored whether the gut microbiome differs between rural and urban populations. Like the variations observed in vaccine responses and immunological profiles, the gut microbiome also differed markedly between these settings. Our study revealed that rural-living Tanzanians exhibited higher within-sample microbial diversity, evidenced by greater Shannon diversity, a higher number of observed Amplicon Sequence Variants (ASVs), and a greater variety of observed genera compared to their urban counterparts. Compositionally, rural individuals were enriched with Succinivibrio, Treponema, and Prevotellaceae (including Prevotella and Alloprevotella), while urban individuals showed higher levels of *Bacteroides* and *Parabacteroides*. This indicates that rural Tanzanians have a

more diverse and balanced microbial community and that there are distinct differences in gut microbiota composition between rural and urban settings. Several factors could explain this difference, including diet, environmental factors, and lifestyle factors such as housing and animal contact.

Our findings align with broader research in this field, which consistently shows that rural populations possess more diverse gut microbiomes[46-50]. Like our findings, other studies in rural settings in Africa show higher microbial diversity, including a predominance of Prevotella[50]. These microbes are known to produce short-chain fatty acids (SCFAs) like butyrate and propionate, which play a crucial role in maintaining gut barrier integrity and modulating immune responses, potentially enhancing vaccine efficacy[51-54]. However, particularly in low- and middle-income settings, counterintuitive findings have been reported in children who received Rotavirus vaccines, where higher microbial diversity was associated with poor vaccine efficacy[55]. This could occur due to increased competition among microbial species, where the abundance of beneficial microbes essential for optimal vaccine response is affected, or due to the presence of potentially pathogenic species, which can cause immune overstimulation or exhaustion, resulting in a lower vaccine response (Levine, 2010; Lynn, 2022). For example, most oral vaccines rely on the gut's immune system to elicit an immune response, but the presence of more diverse microbes or a higher burden of enteric pathogens may reduce the vaccine performance by competing for cell entry or receptor binding[56]. This suggests that not only the quantity but also the balanced quality of the microbial community is crucial for the survival of beneficial bacteria, which act as natural adjuvants or sources of short-chain fatty acids.

Urban populations, on the other hand, showed an increased abundance of Bacteroides[57, 58] , a strain that has been observed to displace *Prevotella* across generations[59, 60] . These microbiota compositions are linked to reduced microbial diversity and increased inflammation, which, in a vaccine context-dependent manner, can either enhance or impair immune function and vaccine efficacy[61, 62]. The enrichment of *Succinivibrio, Treponema, and Prevotellaceae* among rural Tanzanians echoes findings from studies on traditional populations like the Hadza of Tanzania and immigrant populations in the USA[49, 60]

Like other low- and middle-income countries, Tanzania is undergoing rapid urbanization, impacting lifestyle choices and environmental exposure regardless of whether people live in

rural or urban areas. For example, rural individuals now have easier access to fast food or may adopt urban lifestyles by cooking or consuming highly refined foods. These lifestyle changes, along with environmental changes, can influence the gut microbiome and, consequently, the immune system. Our study found that genera associated with urban living were notably enriched for BloSSUM (bloom or selected in societies of urbanization/modernization) taxa, indicating their adaptation to urbanized environments. This aligns with existing literature showing that urbanization leads to the enrichment of microbial taxa adapted to processed foods and reduced microbial diversity typically found in urban settings[59, 63, 64]. In contrast, genera linked to rural living were not significantly enriched for VANISH (volatile and/or associated negatively with industrialized societies of humans) taxa, suggesting that rural individuals might be undergoing a transition, already departing from traditional microbial profiles and reflecting early signs of exposure to urban lifestyles. This observation is supported by studies showing that rural populations exposed to urbanized lifestyles exhibit a decrease in microbiome diversity and an increase in taxa associated with urbanization[48, 65, 66]. This transitional microbiome state in rural populations might suggest an intermediate immune response to vaccines, potentially more effective than that of urban populations but not as robust as that of traditional rural microbiomes.

In the same **chapter 5,** we assessed the impacts of dietary habits on microbiota composition. The study of dietary habits and their impact on gut microbiota composition provides important insights into how diet might influence vaccine efficacy. Samples were clustered into two community state types (CSTs): CST1 was enriched for *Prevotella,* common in rural individuals, and CST2 was enriched for Bacteroides, common in urban individuals. Interestingly, ~ 93% of rural individuals remained in CST1, but urban individuals were split between CST1 ~ 63% and CST2 ~37%. Urban individuals harbouring *Prevotella* were classified as "rural-like urban" (urban+CST1), while those harbouring *Bacteroides* were classified as "urban" (urban+CST2). This classification highlighted the heterogeneity among individuals, regardless of their living locations. Based on dietary habits, individuals with rural-like gut microbiota (CST1) consumed more fibre-rich and carbohydrate-rich diets, such as *ugali* (stiff porridge) and locally made beer (*mbege*), and fewer starch-based foods like potatoes and rice. *Ugali* in rural areas is made from whole grain maize flour, whereas in urban areas it is made from refined maize flour, making it different in content; the rural *ugali* retains the bran, germ, and endosperm, while the urban version has only the endosperm. The locally

made beer, *mbege*, is made from fermented banana and finger millet; fermented foods are created by encouraging the growth of beneficial microbes and the enzymatic breakdown of food elements[67]. These food contents have been shown to result in the bioavailability of nutrients, flavonoids, tannins, phytochemicals, bioactive compounds, and microbial metabolites that are normally consumed as a rich source of probiotic microbes and are thought to act as immunomodulatory compounds[68, 69]. Indeed, findings indicate that certain fermented foods have the potential to promote gut immunity[68, 69]. Therefore, the influence of fermented products, though they promise to modulate vaccine responses, warrants local well-designed interventional studies. Regarding 'rural-like urban' individuals (urban+CST1), they consumed more beans and peas, which was different from urban individuals (urban+CST2) who consumed more rice, potatoes (chips), and refined *ugali*. The intermediate state of the rural-like urban group suggests that the continued consumption of traditional, fiber-rich foods like beans and peas, supports a more diverse gut microbiota and immune responses similar to rural populations, but with components of urban microbiome.

Apart from diet, studies in animals have shown that living environments including housing significantly influence the immune system. For instance, housing plays a crucial role in shaping the gut microbiome of pigs early in life[70]. Individuals sharing an environment, such as cohabiting parents, exhibit 50% less immunological variation compared to individuals in the broader population[71]. Shared environments lead to similar immune profiles[72] and gut microbiota among cohabiting individuals and their animals(e.g., dogs)[73]. Together, these findings indicate that lifestyle factors significantly shape the immune system, potentially due to shared environmental factors such as pathogens, microbiomes, animal contact and dietary patterns. Therefore, information about lifestyle factors can help explain the factors not accounted for when using the rural-urban dichotomy. Lifestyle factors can also better enhance our understanding of the complexity of human-environment interactions, providing detailed insights into human life.

Gut microbiome and antibody waning.

In **Chapter 5**, we assessed how the gut microbiome is associated with yellow fever antibody titers over time. As mentioned already, rural individuals, overall, had higher antibody titers compared to urban individuals. We also found that individuals with a rural-like gut microbiome (CST1) living in rural areas had higher antibody levels compared to those with an

urban-like microbiome (CST2) living in urban settings. Interestingly, individuals characterized by a rural microbiome while living in an urban environment ("urban-like rural" or urban+CST1) also had higher antibody levels than those with an urban-like microbiome (CST2). However, these "urban-like rural" individuals experienced faster antibody waning compared to the other groups. This accelerated waning among individuals with rural-like microbiome characteristics in an urban setting could be due to the reduction of certain beneficial microbes, which may impair the production of immune-enhancing metabolites like short-chain fatty acids (SCFAs). SCFAs are important for antibody production, mutation, and maturation. To the best of our knowledge, no studies have specifically investigated the association between the gut microbiome and the yellow fever vaccine. However, studies on other parenteral mRNA vaccines have shown that the gut microbiome can influence vaccine response, potentially by facilitating SCFA production or acting as a natural adjuvant. For example, the presence of bacterial species such as *Eubacterium rectale* and *Roseburia faecis*, which produce butyrate (an SCFA that acts as a natural adjuvant), has been associated with increased immunogenicity in recipients of the BNT162b2 mRNA COVID-19 vaccine[74]. Similarly, *Bifidobacterium adolescentis* has been linked to higher neutralizing antibodies in CoronaVac vaccine recipients, enhancing immune protection through enriched carbohydrate metabolic pathways[74]. Among children who received fermented formula milk alongside the poliovirus vaccine, antibody levels correlated with the presence of *Bifidobacterium longum* or *Bifidobacterium infantis[75]*. Therefore, the reduction of some of these beneficial bacteria may contribute to the observed pattern. Metagenomic analysis could offer a more detailed understanding of the pathways involved. The faster reduction of antibodies in the 'urban-like rural' group is particularly noteworthy. Given that the Plaque Reduction Neutralization Test (PRNT) is not selective for IgG alone, the early effects of other antibodies, such as IgM, may also be observed. If this is the case, individuals experiencing a more rapid decline in antibody levels may have generated higher amounts of IgM initially, which then decreased over time. Indeed, IgM typically begins to wane around 15 days post-vaccination and can reach nearly undetectable levels by 18 months[76]. Isotype-specific ELISA could be utilized to determine whether there are differences in the induction of IgG and IgM. Waning is the key determinant of the need for and frequency of revaccinations. Waning is also important in general immunization programs, especially in endemic areas, because if antibody levels decline too quickly, it may be unlikely to reach herd immunity. Faster yellow fever antibody waning has been observed in rural or in LMIC when comparing rural and urban populations[26] or

between low- and middle-income countries and high-income countries[28]. However, this observation has not been directly linked to the gut microbiome in the context of yellow fever vaccine studies. It is important to note that for vaccines like the CoronaVac, the baseline gut microbiome has been able to predict vaccine immunogenicity, particularly in individuals with a high abundance of *Bifidobacterium adolescentis*, *Bifidobacterium bifidum*, and *Roseburia faecis* [77]. It is also important to note that while humoral immunity is crucial, effective immunity requires both humoral and cellular responses for complete protection against disease. Therefore, it is essential to assess not only antibody levels but also the strength of cellular immune responses.

Future perspectives

Exploring the role of diet on the microbiota and vaccine efficacy

Research into the microbiota's role in enhancing vaccine efficacy is an emerging and promising field. There is a complex relationship between diet, microbiota, and the immune system. Findings suggest that metabolites produced by the microbiota, along with specific dietary components, such as those found in fermented food diets, can modulate immune responses[78]. To optimize immunization strategies, future research might focus on identifying pathways and mechanisms through which the microbiota influences vaccine responses. In our research, we found an association between diets rich in fibers or complex carbohydrates can influence the gut microbiome. A promising approach could involve leveraging locally available diets, such as fermented local beverages in Tanzania, which have been shown to have anti-inflammatory effects[41].

Other dietary options, such as fermented milk, have shown promising potential in enhancing vaccine responses, particularly for influenza vaccines[79] and Salmonella typhi Ty21a[80]. This opens the door for more rigorous exploration through well-designed, controlled trials. By including larger, more diverse populations and evaluating its effects across a broader range of vaccines, we can better understand the role of fermented local beverages in boosting immune responses. In Tanzania, An ongoing study is investigating the impact of traditional plant-based diets and fermented foods on alleviating immune metabolic dysregulation and enhancing vaccine response in overweight and obese individuals[81]. In this study, one group receives a fermented banana beverage, another follows a high plant-based diet, and a third consumes a

normal diet[81]. These dietary interventions are administered alongside various vaccines to assess whether they can improve immune responses. While this study has the potential to offer valuable insights, its results are still pending. A limitation of our current study is the absence of dietary intervention, and we are currently awaiting results from the Tanzania study, the study in which participants, who received intervention are obese individuals, limiting generalization[81]. Therefore, future research should aim to address this gap by integrating dietary modifications alongside vaccination in a controlled cohort of normal-weight, healthy individuals. Such an approach would facilitate the identification of specific dietary compounds or metabolites that may modulate the immune system and enhance vaccine efficacy. Longitudinal studies are particularly well-suited for this purpose, as they can provide comprehensive insights into how various dietary factors influence the gut microbiome and, consequently, impact immune responses to vaccines.

Investigating the role of hypoxia-inducible factors in immune function and vaccine response at high altitude.

High-altitude hypoxia induces hypoxia-inducible factors (HIFs), HIFs affect both innate and adaptive immune cells, including antigen-presenting cells, T-cells, and B lymphocytes, thereby altering their phenotype and function[39]. However, the role of HIFs in vaccine responses remains largely unexplored in humans. HIFs are known to cause significant metabolic and phenotypic changes in B-cells, boosting B-cell differentiation and enhancing CD4+ T-cell function[40]. These changes promote IFN-γ or IL-4 cytokine production[40], which is crucial for antibody production and class switching in B-cells, with the possibility of supporting robust humoral immunity. Studies in animals have demonstrated that HIFs in CD4+ T-cells enhance glycolysis, promote cytokine production, and regulate T-cell subsets, all of which are vital for effective immune responses[40].

To better understand the effects of high altitude on immune function, it would be valuable to measure HIF levels in blood samples collected from different altitude rural areas as well as different altitude urban areas. By assessing whether there are statistically significant differences in HIF expression between these populations, researchers could gain insights into how environmental factors like altitude influence immune responses. This could have important implications for tailoring public health strategies and vaccination programs in high-altitude regions. Additionally, understanding the role of HIFs in immune modulation could

open up new avenues for therapeutic interventions that harness these pathways to enhance the immune response to vaccines.

The importance of inclusive research

Our study underscores the crucial need for inclusive research that considers the diversity of populations, particularly in understanding variations in immune responses and vaccine effectiveness. It is increasingly evident that these variations extend beyond the differences observed between high-income countries (HICs) and low- and middle-income countries (LMICs)[10, 82]; significant differences are also present within geographically proximate regions, such as urban versus rural settings. As demonstrated by our findings, populations living only an hour apart can exhibit striking differences in immunological profiles and gut microbiome composition, as well as subtle but notable differences in vaccine immunogenicity.

Nevertheless, our study was limited by its focus on specific populations and regions, examining only two out of five districts in the region. This indicates the need for broader research that includes a wider range of populations exposed to diverse environmental conditions. Such research is vital not only for deepening our understanding of how various factors influence immune profiles and vaccine responses but also for informing public health strategies that address health disparities linked to distinct environments. Ultimately, this approach could lead to more effective and equitable vaccination strategies, particularly through the tailoring of vaccine formulations and immunization schedules to specific environmental exposures.

Prioritizing immune variation or its drivers

Deciding whether to focus future research on immune variation itself or the underlying drivers, such as microbiome and metabolome variations, is pivotal for advancing our understanding of immune responses to vaccines. Investigating immune variation directly provides immediate insights into how immune responses differ among individuals and populations, which is highly relevant for developing targeted immunization strategies. This approach helps identify specific immune profiles associated with better vaccine responses or increased susceptibility to infections, allowing for tailored interventions in diverse settings. However, this strategy may overlook the root causes of these immune differences. Without understanding the drivers of

immune variation, sustainable solutions to address differences in immune responses might remain elusive.

On the other hand, focusing on the drivers of immune variation, such as the microbiome, lifestyle factors, and metabolome, offers a more comprehensive understanding of the factors influencing immune responses. These drivers interact with the host immune system in complex ways, affecting everything from immune development to disease progression. For instance, different microbes can harbour the same metabolic pathways, leading to similar biological outcomes, and different environmental drivers can produce the same downstream immune effects. This redundancy approach suggests that studying these drivers could help uncover universal mechanisms underlying immune responses, potentially leading to broad-spectrum interventions that are effective across diverse populations. However, this approach is not without its challenges. The complexity of, for example, microbiome and metabolome, coupled with their interactions with various environmental factors, makes it difficult to pinpoint specific drivers of immune variation. Moreover, the same microbial or metabolic changes can have different effects depending on the host's genetic background, health status, and environmental exposures, complicating the translation of these findings into actionable public health strategies. Again, a more balanced approach, as I often refer to in the discussion of this thesis, that integrates both immune variation and its drivers may provide the most robust framework for understanding and enhancing immune responses across populations.

Combining advanced technology, data analysis, and integration to harness the individual studies

Leveraging advanced technologies such as transcriptomics, metabolomics, epigenetic analyses at the single-cell level, and high-dimensional cytometry presents unprecedented opportunities to study immune variation and vaccine-specific immune responses in greater depth and breadth. These technologies can uncover new immunological pathways and networks, paving the way for designing more effective vaccines.

To maximize the potential of these advanced tools, it is crucial to utilize and expand international collaborative networks, such as Hypovax Global (hypovax.org), which can provide access to cutting-edge technologies and expertise. Additionally, investing in training and capacity-building initiatives will empower local scientists to analyze and interpret complex

datasets. Establishing local data servers or data sharing points and other data-sharing platforms can address the challenges posed by the relative lack of infrastructure, that enables large-scale data storage, processing, and analysis. This way, new PhD students can be recruited easily and PhD students who finish their training can continue their work and contribute toward understanding the variation in vaccine response. Moreover, standardizing protocols and harmonizing data formats will ensure consistency and facilitate meta-analyses across studies. Access to user-friendly bioinformatics tools, particularly open-source software with localized adaptations, will further enhance the ability of researchers in LMICs to conduct advanced data analyses, ultimately contributing to more robust and impactful scientific outcomes.

Conclusion

In conclusion, the work presented in this thesis contributes to the expanding body of literature demonstrating that immune system variability exists between populations, as well as differences in vaccine efficacy/immunogenicity, particularly when comparing rural and urban populations. This research, uniquely based in Africa, highlights the importance of lifestyle factors such as housing, asset ownership, and dietary history as key variables in understanding immune system variation. These findings address gaps that a simplistic rural-urban dichotomy would have missed. If applied carefully, a lifestyle score can provide immunologists, vaccinologists, public health experts, and researchers with a deeper understanding of how these factors influence immune function, and vaccine responses.

Furthermore, this thesis underscores the context-dependent nature of vaccine responses, emphasizing the need for bidirectional hypotheses. This approach allows for more precise mapping of the factors influencing vaccine hypo-responsiveness. Additionally, the association between the gut microbiome and the faster, stronger antibody waning observed in individuals with urban but rural-like characteristics suggests that the microbiome plays a crucial role in immune response regulation. These findings, particularly the unexpectedly higher antibody levels in rural populations and the accelerated antibody waning in urban individuals with rural-like traits call for further research into the role of the microbiome.

References:

1. Corstjens, P.L.A.M., et al., *Circulating Anodic Antigen (CAA): A Highly Sensitive Diagnostic Biomarker to Detect Active Infections-Improvement and Use during SCORE.* American Journal of Tropical Medicine and Hygiene, 2020. **103**(1): p. 50-57.
2. Poggensee, G., et al., *A six-year follow-up of schoolchildren for urinary and intestinal schistosomiasis and soil-transmitted helminthiasis in Northern Tanzania.* Acta Tropica, 2005. **93**(2): p. 131-140.
3. Dunn, J.C., et al., *The increased sensitivity of qPCR in comparison to Kato-Katz is required for the accurate assessment of the prevalence of soil-transmitted helminth infection in settings that have received multiple rounds of mass drug administration.* Parasites & Vectors, 2020. **13**(1).
4. Oettle, R.C. and S. Wilson, *The Interdependence between Schistosome Transmission and Protective Immunity.* Tropical Medicine and Infectious Disease, 2017. **2**(3).
5. Nikolay, B., S.J. Brooker, and R.L. Pullan, *Sensitivity of diagnostic tests for human soil-transmitted helminth infections: a meta-analysis in the absence of a true gold standard.* International Journal for Parasitology, 2014. **44**(11): p. 765-774.
6. de Ruiter, K., et al., *Helminth infections drive heterogeneity in human type 2 and regulatory cells.* Science Translational Medicine, 2020. **12**(524).
7. Wammes, L.J., et al., *Community deworming alleviates geohelminth-induced immune hyporesponsiveness.* Proceedings of the National Academy of Sciences of the United States of America, 2016. **113**(44): p. 12526-12531.
8. Houlder, E.L., et al., *Early symptom-associated inflammatory responses shift to type 2 responses in controlled human schistosome infection.* Science Immunology, 2024. **9**(97).
9. Tweyongyere, R., et al., *Effect of Praziquantel Treatment during Pregnancy on Cytokine Responses to Schistosome Antigens: Results of a Randomized, Placebo-Controlled Trial.* Journal of Infectious Diseases, 2008. **198**(12): p. 1870-1879.
10. Mbow, M., et al., *Changes in immunological profile as a function of urbanization and lifestyle.* Immunology, 2014. **143**(4): p. 569-577.
11. Yan, Z., et al., *Aging and CMV discordance are associated with increased immune diversity between monozygotic twins.* Immunity & Ageing, 2021. **18**(1).
12. Wikel, S.K., *Modulation of the host immune system by ectoparasitic arthropods - Blood-feeding and tissue-dwelling arthropods manipulate host defenses to their advantage.* Bioscience, 1999. **49**(4): p. 311-320.
13. Brown, R., et al., *Fungal Toxins and Host Immune Responses.* Frontiers in Microbiology, 2021. **12**.
14. Harding, C.F., et al., *Mold inhalation causes innate immune activation, neural, cognitive and emotional dysfunction.* Brain Behavior and Immunity, 2020. **87**: p. 218-228.
15. Mrimi, E.C., et al., *Correlation of Cytokines with Parasitic Infections, Undernutrition and Micronutrient Deficiency among Schoolchildren in*

Rural Tanzania: A Cross-Sectional Study. Nutrients, 2023. **15**(8).

16. Alam, I., A. Larbi, and G. Pawelec, *Nutritional status influences peripheral immune cell phenotypes in healthy men in rural Pakistan.* Immunity & Ageing, 2012. **9**.
17. Fergusson, J.R., et al., *CD161 CD8+T cells: a novel population of highly functional, memory CD8+T cells enriched within the gut.* Mucosal Immunology, 2016. **9**(2): p. 401-413.
18. Ito, M., et al., *Killer cell lectin-like receptor G1 binds three members of the classical cadherin family to inhibit NK cell cytotoxicity.* Journal of Experimental Medicine, 2006. **203**(2): p. 289-295.
19. Robbins, S.H., et al., *Cutting edge: Inhibitory functions of the killer cell lectin-like receptor G1 molecule during the activation of mouse NK cells.* Journal of Immunology, 2002. **168**(6): p. 2585-2589.
20. Zhang, Y.K., et al., *The role of KLRG1: a novel biomarker and new therapeutic target.* Cell Communication and Signaling, 2024. **22**(1).
21. Shimwela, M., et al., *Asthma prevalence, knowledge, and perceptions among secondary school pupils in rural and urban coastal districts in Tanzania.* Bmc Public Health, 2014. **14**.
22. Rodriguez, A., et al., *Urbanisation and asthma in low-income and middle-income countries: a systematic review of the urban-rural differences in asthma prevalence.* Thorax, 2019. **74**(11): p. 1020-1030.
23. Monath, T.P., et al., *Yellow Fever 17D Vaccine Safety and Immunogenicity in the Elderly.* Human Vaccines, 2005. **1**(5): p. 207-214.
24. Monath, T.P., et al., *Comparative safety and immunogenicity of two yellow fever 17D vaccines (Arilvax and YF-VAX) in a Phase III multicenter, double-blind clinical trial.* American Journal of Tropical Medicine and Hygiene, 2002. **66**(5): p. 533-541.
25. Rosenstein, M.D., et al., *Long-term immunity after a single yellow fever vaccination in travelers vaccinated at 60 years or older: A 10-year follow-up study.* Journal of Travel Medicine, 2021. **28**(8).
26. Domingo, C., et al., *Long-term immunity against yellow fever in children vaccinated during infancy: a longitudinal cohort study.* Lancet Infectious Diseases, 2019. **19**(12): p. 1363-1370.
27. Nkurunungi, G.a.N., Jacent and Natukunda, Agnes and Zirimenya, Ludoviko and Walusimbi, Bridgious and Zziwa, Christopher and Ninsiima, Caroline and Kabagenyi, Joyce and Kabuubi, Prossy Nakawungu and van Dam, Govert J. and Corstjens, Paul and Kayiwa, John and Kizza, Moses and Mutebe, Alex and Nakazibwe, Esther and Akello, Florence Ateng and Sewankambo, Moses and Kiwanuka, Samuel and Cose, Stephen and Wajja, Anne and Kaleebu, Pontiano and Webb, Emily and Elliott, Alison, *The Effect of Intensive Praziquantel Treatment on Vaccine-Specific Responses Among Schoolchildren in Ugandan Schistosomiasis-Endemic Islands: Results of the Popvac a Randomised, Controlled Trial.* SSRN, 2023.
28. Muyanja, E., et al., *Immune activation alters cellular and humoral responses to*

yellow fever 17D vaccine (vol 124, pg 3147, 2014). Journal of Clinical Investigation, 2014. **124**(10): p. 4669-4669.

29. Stelekati, E. and E.J. Wherry, *Chronic Bystander Infections and Immunity to Unrelated Antigens.* Cell Host & Microbe, 2012. **12**(4): p. 458-469.
30. Schramm, G. and H. Haas, *Th2 immune response against infection.* Microbes and Infection, 2010. **12**(12-13): p. 881-888.
31. Vlasova, A.N., et al., *How the gut microbiome regulates host immune responses to viral vaccines.* Current Opinion in Virology, 2019. **37**: p. 16-25.
32. Bernard Charlery de la Masselière, F.B., Bénédicte Thibaud et Rémi Benos, *Revisiting the rural-urban linkages in East Africa: Continuity or breakdown in the spatial model of rural development? The case of the Kilimanjaro region in Tanzania.* OpenEdition Journals, 2020.
33. Tesfaye, T.S., F. Tessema, and H. Jarso, *Prevalence of Anemia and Associated Factors Among "Apparently Healthy" Urban and Rural Residents in Ethiopia: A Comparative Cross-Sectional Study.* Journal of Blood Medicine, 2020. **11**: p. 89-96.
34. Kitange, H.M., et al., *Anemia Is a Major Public-Health Problem in Tanzania.* Health Policy and Planning, 1993. **8**(4): p. 413-424.
35. Arnett, D. *Topographic Map of Mrawi, Uru Kaskazini, Moshi, Tanzania.* 2024; Available from: https://elevationmap.net/contact.
36. Cho, S.H., et al., *Germinal centre hypoxia and regulation of antibody qualities by a hypoxia response system.* Nature, 2016. **537**(7619): p. 234-+.
37. McGettrick, A.F. and L.A.J. O'Neill, *The Role of HIF in Immunity and Inflammation.* Cell Metabolism, 2020. **32**(4): p. 524-536.
38. Abbott, R.K., et al., *Germinal Center Hypoxia Potentiates Immunoglobulin Class Switch Recombination.* Journal of Immunology, 2016. **197**(10): p. 4014-4020.
39. Taylor, C.T. and C.C. Scholz, *The effect of HIF on metabolism and immunity.* Nature Reviews Nephrology, 2022. **18**(9): p. 573-587.
40. Cho, S.H., et al., *Hypoxia-inducible factors in CD4 T cells promote metabolism, switch cytokine secretion, and T cell help in humoral immunity.* Proceedings of the National Academy of Sciences of the United States of America, 2019. **116**(18): p. 8975-8984.
41. Temba, G.S., et al., *Urban living in healthy Tanzanians is associated with an inflammatory status driven by dietary and metabolic changes.* Nature Immunology, 2021. **22**(3): p. 287-+.
42. Boahen, C.K., et al., *A functional genomics approach in Tanzanian population identifies distinct genetic regulators of cytokine production compared to European population.* American Journal of Human Genetics, 2022. **109**(3): p. 471-485.
43. van Dorst, M., et al., *Immunological factors linked to geographical variation in vaccine responses.* Nat Rev Immunol, 2023.
44. Leitner, W.W., et al., *Type I Interferons are essential for the efficacy of replicase-based DNA vaccines.* Vaccine, 2006. **24**(24): p. 5110-5118.

45. McNab, F., et al., *Type I interferons in infectious disease.* Nature Reviews Immunology, 2015. **15**(2): p. 87-103.
46. Clemente, J.C., et al., *The microbiome of uncontacted Amerindians.* Science Advances, 2015. **1**(3).
47. Gomez, A., et al., *Gut Microbiome of Coexisting BaAka Pygmies and Bantu Reflects Gradients of Traditional Subsistence Patterns.* Cell Reports, 2016. **14**(9): p. 2142-2153.
48. Schnorr, S.L., et al., *Gut microbiome of the Hadza hunter-gatherers.* Nature Communications, 2014. **5**.
49. Smits, S.A., et al., *Seasonal cycling in the gut microbiome of the Hadza hunter-gatherers of Tanzania.* Science, 2017. **357**(6353): p. 802-+.
50. Yatsunenko, T., et al., *Human gut microbiome viewed across age and geography.* Nature, 2012. **486**(7402): p. 222-+.
51. Mirzaei, R., et al., *Dual role of microbiota-derived short-chain fatty acids on host and pathogen.* Biomedicine & Pharmacotherapy, 2022. **145**.
52. Wu, Q., et al., *Short-Chain Fatty Acids Alleviate Vancomycin-Caused Humoral Immunity Attenuation in Rabies-Vaccinated Mice by Promoting the Generation of Plasma Cells via Akt-mTOR Pathway.* Journal of Virology, 2023. **97**(7).
53. Lynn, D.J., et al., *Modulation of immune responses to vaccination by the microbiota: implications and potential mechanisms.* Nature Reviews Immunology, 2022. **22**(1): p. 33-46.
54. van der Hee, B. and J.M. Wells, *Microbial Regulation of Host Physiology by Short-chain Fatty Acids.* Trends in Microbiology, 2021. **29**(8): p. 700-712.
55. Cunningham-Oakes, E., et al., *Increased bacterial taxonomic and functional diversity is associated with impaired rotavirus vaccine immunogenicity in infants from India and Malawi.* Bmc Microbiology, 2023. **23**(1).
56. Parker, E.P.K., et al., *Causes of impaired oral vaccine efficacy in developing countries.* Future Microbiology, 2018. **13**(1): p. 97-118.
57. Strazar, M., et al., *Gut microbiome-mediated metabolism effects on immunity in rural and urban African populations (vol 12, 4845, 2021).* Nature Communications, 2021. **12**(1).
58. Tamburini, F.B., et al., *Short- and long-read metagenomics of urban and rural South African gut microbiomes reveal a transitional composition and undescribed taxa.* Nature Communications, 2022. **13**(1).
59. Vangay, P., et al., *US Immigration Westernizes the Human Gut Microbiome.* Cell, 2018. **175**(4): p. 962-+.
60. Truter, M., et al., *Documenting the diversity of the Namibian Ju|'hoansi intestinal microbiome.* Cell Reports, 2024. **43**(2).
61. Hagan, T., et al., *Antibiotics-Driven Gut Microbiome Perturbation Alters Immunity to Vaccines in Humans.* Cell, 2019. **178**(6): p. 1313-+.
62. Huda, M.N., et al., *Stool Microbiota and Vaccine Responses of Infants.* Pediatrics, 2014. **134**(2): p. E362-E372.
63. Sun, S., et al., *Loss of Novel Diversity in Human Gut Microbiota Associated with Ongoing Urbanization in China.* Msystems, 2022. **7**(4).

64. Zuo, T., et al., *Urbanization and the gut microbiota in health and inflammatory bowel disease.* Nature Reviews Gastroenterology & Hepatology, 2018. **15**(7): p. 440-452.
65. Rampelli, S., et al., *Metagenome Sequencing of the Hadza Hunter-Gatherer Gut Microbiota.* Current Biology, 2015. **25**(13): p. 1682-1693.
66. Morandini, F., et al., *Urbanization associates with restricted gut microbiome diversity and delayed maturation in infants.* Iscience, 2023. **26**(11).
67. Marco, M.L., et al., *The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on fermented foods.* Nature Reviews Gastroenterology & Hepatology, 2021. **18**(3): p. 196-208.
68. Marco, M.L., et al., *Health benefits of fermented foods: microbiota and beyond.* Current Opinion in Biotechnology, 2017. **44**: p. 94-102.
69. Ashraf, R. and N.P. Shah, *Immune System Stimulation by Probiotic Microorganisms.* Critical Reviews in Food Science and Nutrition, 2014. **54**(7): p. 938-956.
70. Wen, C.F., et al., *Environmentally enriched housing conditions affect pig welfare, immune system and gut microbiota in early life.* Animal Microbiome, 2021. **3**(1).
71. Ramasubramanian, R., et al., *Cohabitation as a determinant of adaptive and innate immune cell profiles: Findings from the Health and Retirement Study.* Brain, Behavior, & Immunity - Health, 2023. **33**.
72. Carr, E.J., et al., *The cellular composition of the human immune system is shaped by age and cohabitation.* Nature Immunology, 2016. **17**(4): p. 461-+.
73. Song, S.J., et al., *Cohabiting family members share microbiota with one another and with their dogs.* Elife, 2013. **2**.
74. Ng, S.C., et al., *Gut microbiota composition is associated with SARS-CoV-2 vaccine immunogenicity and adverse events.* Gut, 2022. **71**(6): p. 1106-+.
75. Mullié, C., et al., *Increased poliovirus-specific intestinal antibody response coincides with promotion of and in infants:: A randomized, double-blind, placebo-controlled trial.* Pediatric Research, 2004. **56**(5): p. 791-795.
76. Pulendran, B., *Learning immunology from the yellow fever vaccine: innate immunity to systems vaccinology.* Nature Reviews Immunology, 2009. **9**(10): p. 741-747.
77. Peng, Y., et al., *Baseline gut microbiota and metabolome predict durable immunogenicity to SARS-CoV-2 vaccines.* Signal Transduction and Targeted Therapy, 2023. **8**(1).
78. Wastyk, H.C., et al., *Gut-microbiota-targeted diets modulate human immune status.* Cell, 2021. **184**(16): p. 4137-+.
79. Boge, T., et al., *A probiotic fermented dairy drink improves antibody response to influenza vaccination in the elderly in two randomised controlled trials.* Vaccine, 2009. **27**(41): p. 5677-5684.
80. Linkamster, H., et al., *Modulation of a Specific Humoral Immune-Response and Changes in Intestinal Flora Mediated through Fermented Milk Intake (Vol 10, Pg 55, 1994).* Fems Immunology and Medical Microbiology, 1995. **12**(3-4): p. 273-273.

81. ISRCTN68451814, *The effect of traditional diets on immune regulation in obese adults in Tanzania*. 2023: ISRCTNregistry
82. Labuda, L.A., et al., *A Praziquantel Treatment Study of Immune and Transcriptome Profiles in -Infected Gabonese Schoolchildren.* Journal of Infectious Diseases, 2020. **222**(12): p. 2103-2113.

Appendices

Nederlandse Samenvatting:

Het overkoepelende doel van dit proefschrift was het onderzoeken van de factoren die samenhangen met variaties in het immuunsysteem en verschillen in vaccin responsen bij Tanzaniaanse volwassenen, met behulp van geavanceerde technologieën zoals single-cell-analyse via massa cytometrie en 16S rRNA-sequencing. Om dit te bereiken voerden we een literatuurstudie en drie verschillende veldonderzoeken uit (twee cross-sectionele studies en één studie met longitudinale follow-up).

Allereerst voerden we een literatuurstudie uit, gevolgd door twee cross-sectionele studies. De eerste van deze twee richtte zich op het evalueren van de prevalentie en diagnostische nauwkeurigheid van hulpmiddelen die worden gebruikt voor de diagnose van schistosomiasis in een landelijke omgeving. Deze studie betrof meer dan 500 schoolgaande kinderen en leverde cruciale inzichten op in de prevalentie van de ziekte in Tanzania en in de effectiviteit van diagnostische methoden in een omgeving met beperkte middelen. De tweede cross-sectionele studie had als doel de immunologische profielen van personen uit landelijke en stedelijke gebieden te vergelijken en factoren te identificeren die bijdragen aan immuunvariatie. In deze studie werden deelnemers geworven uit vier verschillende locaties - twee landelijke en twee stedelijke - waar bloed-, ontlastings- en urinemonsters werden verzameld. Gedetailleerde vragenlijsten registreerden individuele leefstijlfactoren zoals sociaal-economische status, dieet en omgevingsblootstelling, wat hielp bij het verduidelijken van de intrinsieke en extrinsieke oorzaken van immuunvariatie.

Een derde studie, een longitudinale cohortstudie, resulteerde al in twee wetenschappelijke artikelen. Deze studie betrof de follow-up van personen uit twee van de geselecteerde onderzoekslocaties, één landelijke en één stedelijke. In totaal werden 185 deelnemers geworven, gelijkmatig verdeeld tussen landelijke en stedelijke gebieden. Om de factoren te onderzoeken die de vaccinrespons beïnvloeden, werd beide groepen het gele koorts-vaccin toegediend. Biologische monsters (bloed, ontlasting, urine) werden verzameld op meerdere tijdstippen: vóór de vaccinatie en op dag 2, 7, 14, 28, 56, 90 en 178 na vaccinatie. Daarnaast werd gedetailleerde informatie over de leefstijl verzameld via vragenlijsten, met gegevens over sociaal-economische factoren, dieet en andere relevante variabelen. Geavanceerde single-cell-technologie, zoals massacytometrie, hielp bij het in kaart brengen van immuun-cel profielen

met hoge resolutie, terwijl 16S rRNA-sequencing inzicht bood in de samenstelling van het microbioom.

Belangrijkste bevindingen per hoofdstuk:

Hoofdstuk 1: Hier introduceerden we de factoren die samenhangen met variaties in het immuunsysteem en de verschillen in immuunresponsen op vaccins. Daarnaast beschreven we de belangrijkste doelstellingen van dit proefschrift, de onderzoeksopzet, de geografische gebieden waar de studies zijn uitgevoerd, en de onderzochte populaties. In **Hoofdstuk 2** bespraken we de aanzienlijke uitdaging die hypo-responsiviteit op vaccins vormt voor de wereldwijde volksgezondheid, met name door de variabiliteit in effectiviteit van vaccins tussen verschillende populaties en geografische regio's. Dit probleem is vooral zichtbaar in landen met een laag of midden inkomen, waar vaccins tegen ziekten zoals malaria, tuberculose (Tb) en rotavirus vaak een verminderde immunogeniciteit en effectiviteit laten zien in vergelijking met landen met een hoog inkomen. We identificeerden meerdere bijdragende factoren aan deze hypo-responsiviteit, waaronder blootstelling aan micro-organismen en parasieten (zoals HIV, CMV, malaria, parasitaire wormen, en omgevings mycobacteriën), variaties in het microbioom (zoals faagdiversiteit en commensale bacteriën), en de aanwezigheid van pro- en anti-inflammatoire metabolieten (bijvoorbeeld flavonen). We onderzochten mogelijke immunologische mechanismen achter verminderde vaccin responsen, waaronder pre-existente immuniteit, persisterende immuunactivatie, immuun uitputting, en veranderingen in weefsel micro-omgevingen, zoals in de lymfeklieren. Ook bespraken we potentiële strategieën om vaccin responsen te verbeteren, zoals het aanpassen van adjuvantia, het wijzigen van vaccinatie regimes, het verminderen van ontstekingsreacties, en het gebruik van monoklonale antilichamen gericht tegen Th2-cytokines en Treg-cellen.

Hoofdstuk 3: We onderzochten de prevalentie van schistosomiasis bij scholieren in het Mwanga-district, Tanzania, na bijna twee decennia van Mass Drug Administration (MDA) met praziquantel. Hiervoor gebruikten we de Up-Converting Particle Lateral Flow Circulating Anodic Antigen (UCP-LF CAA) test, bekend om zijn hoge gevoeligheid. Daarnaast onderzochten we de Point-of-Care Circulating Cathodic Antigen (POC-CCA) test en de microhematurie-dipstick als diagnostische hulpmiddelen. Onze bevindingen toonden een prevalentie van schistosomiasis van 20,3% op basis van de UCP-LF CAA-test, wat een nauwkeuriger weergave van de ziektelast gaf dan de POC-CCA- en microhematurie-tests.

Onze studie benadrukte de aanhoudende overdracht van schistosomiasis ondanks langdurige MDA-inspanningen en de noodzaak van verbeterde diagnostische tools voor veldgebruik. **Hoofdstuk 4:** We onderzochten de relatie tussen leefstijlfactoren en cellulaire immuunprofielen bij gezonde Tanzaniaanse volwassenen. De leefstijlscores waren gebaseerd op huishoudelijke bezittingen, woonsituatie en dieetgeschiedenis. We vonden significante verschillen in immuuncel frequentie tussen landelijke en stedelijke deelnemers. Landelijke deelnemers vertoonden hogere frequenties van Th2-cellen, atypische geheugen-B-cellen, en CD4+ T-effector geheugencellen. Leefstijlfactoren bleken een significante invloed te hebben op cellulaire immuunprofielen, onafhankelijk van de geografische locatie. **Hoofdstuk 5:** We vergeleken de immunogeniciteit van het gele koorts-vaccin in landelijk en stedelijk Moshi. Immunogeniciteit werd functioneel gemeten met een neutralisatietest, en titers van antilichamen tegen niet-structureel eiwit 1 (NS1) werden gemeten met ELISA. **Hoofdstuk 6:** We onderzochten de samenstelling van het darmmicrobioom bij Tanzaniaanse volwassenen uit landelijke en stedelijke gebieden in relatie tot de antilichaamrespons op het gele koorts-vaccin. We vonden significante verschillen in microbiota samenstelling tussen de twee groepen. Landelijke deelnemers vertoonden een grotere microbiële diversiteit, wat deels werd verklaard door dieetverschillen. **Hoofdstuk 7:** We bespraken de belangrijkste bevindingen van dit proefschrift, met aandacht voor de factoren die bijdragen aan variaties in het immuunsysteem en verschillen in immuunresponsen op vaccins. Tot slot presenteerden we toekomstperspectieven en de algemene conclusies van dit onderzoek.

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Curriculum vitae

Jeremia J. Pyuza (Shadrack) was born on March 31, 1990, at Haydom Lutheran Hospital, near his home village of Marera in what was Iramba District at that time, now Mkalama District of the Singida Region, Tanzania. His educational journey began in this rural setting, where he completed his secondary education in 2009 at Gumanga Secondary School. With a passion for the sciences, he proceeded to Bihawana High School for advanced-level studies, focusing on a science combination.

Driven by a strong interest in health sciences and public service, Jeremia pursued and completed a Bachelor of Science in Medical Laboratory Sciences at Kilimanjaro Christian Medical University College (KCMUCo), now KCMC University in 2015. During his undergraduate years, he became actively involved in public health outreach and discovered a passion for writing. He authored his first book, Laboratory Medicine: The Cornerstone for Evidence-Based Medicine, while still a student.

Determined to bridge laboratory science with clinical practice, Jeremia went on to pursue a Doctor of Medicine (MD) degree at the same university (KCMC University) in 2020. While in medical school, he worked part-time in the Department of Pathology and engaged in various research activities, publishing several scientific papers and authoring two additional books. During his third year of medical studies, he also began an online MBA to build his leadership and interdisciplinary learning. A pivotal moment in Jeremia's career came when he met Prof. Pancras Hogendoorn, then Dean of Leiden University Medical Center (LUMC), during a visit to the Department of Pathology at KCMC Impressed by Jeremia's work and his books, Prof. Pancras encouraged him to develop a research topic and later introduced him to Prof. Maria Yazdanbakhsh, a leading immunologist at LUMC and in the Netherlands, renowned for her research on immune variation across populations in both high-income and low- and middle-income countries.

This collaboration led to a formative research experience on schistosomiasis in Mwanga District, Kilimanjaro, conducted under the supervision of Prof. Sia Msuya as part of his MD training. The promising results of that study paved the way for Jeremia to attend a Half Minor in Global Health at LUMC in the Netherlands, during his fifth year of medical school. Encouraged by Prof. Maria and with the support of the LUMC Global Fund, Jeremia

successfully applied to start a PhD in Leiden shortly after completing his medical degree. During his final year as a medical student, he was recognized as the best finalist in Surgery and received the prestigious Dr. Japhet Urasa Prize. Jeremia also has a Postgraduate Diploma in Family Medicine, which enables him to engage in public health education. He has authored or co-authored several Swahili-language public health books on topics including heart failure, pregnancy, hypertensive disorders, kidney disease, COVID-19, and hepatitis.

Jeremia is currently undergoing specialist training in Anatomic Pathology at KCMC University, where he aims to integrate his immunological expertise into the development of a molecular pathology laboratory. His interests include immune variation and vaccine response, immunopathology, immunohistochemistry, and immunotherapy, with a vision to improve vaccine effectiveness, particularly for infectious diseases, including those involved in cancer prevention and to advance cancer diagnosis and care in Tanzania. He has a coordinating role in the collaborative project between KCMC and LUMC, which includes student exchange programs, half-minor research placements, and PhD-related activities. Jeremia is married to Dr. Alice, and together they are proud parents of two children, Ray-Shadrack and Sarah.

Jeremia J. Pyuza PhD Portfolio:

Department: Leiden University Center for Infectious Diseases (LUCID)

PhD duration: 01/01/2021-15/05/2025

Training activities	**Hours**
Courses	
Introductory course in Epidemiology and Medical statistics London school of hygiene and Tropical Medicine(LSHTM) (2023)	42
Basic methods and reasoning in Biostatistics LUMC (2023)	42
International course in Clinical Epidemiology LUMC (2023)	40
Research regulations and practical implications in the LUMC(2025)	5
Meta analysis LUMC (2024)	30
PhD course in infection and Immunology (LUMC, 2024)	80
Foundations in Virology and Vaccinology Specialization(Coursera, 2025)	39
Certificate Good Clinical Practice(GCP) (2022)	08
Statistical Aspects of clinical trials LUMC (2024)	30
Planning your scientific journey(iBiology, 2022)	15
Let's Experiment : A Guide for Scientists Working at the Bench(iBiology, 2022)	15
Share Your Research: How to Give a Good Talk(iBiology, 2022)	15
Analysis of repeated measurements LUMC (2024)	42
Survival analysis(Advanced Biostatistics) LUMC (2024)	42
Clinical trial analysis, monitoring and presentation(2024)	40
Immunology: Adaptive immune system MOOC (2023)	40
Introduction to R data camp (2024)	04
Introduction to Tidyverse data camp (2024)	04
Intermediate R data camp (2024)	04
Data analysis with R programming MOOC (2024)	04
Introduction to regression data camp (2024)	04
Intermediate regression in R data camp (2024)	04
Building statistical model in R: Linear regression MOOC(2024)	04
Generalized Linear model in R data camp (2024)	04
Hierarchical and mixed effects model in R data camp (2024)	04
Introduction to data visualization with ggplot2 data camp (2024)	04
Data visualization using dplyr and ggplot2 in R MOOC (2024)	04
Introduction to machine learning data camp (2024)	04
Seminar	
Scientific conduct LUMC (2024)	05
Bi weekly department seminar LUMC (2021-2024)	40
Departmental presentation (2021-2024)	09
Conferences, and workshop	
ISBT conference south Africa 2023	28
Hypovax Global workshop Netherlands 2024	28
Innate immunity conference Netherlands 2024	28
FAIR Data conference and Training Leiden (2024)	28
Students supervision	
Half minor 2022	20
Half minor 2023	20
Half minor 2024	20
Field work activities	
Community engagement	40
Participants recruitment	40
Participants follow-up	40
Total hours:	915

List of publications

Publications included in this thesis

Pyuza JJ*, Meulah B*, Hoekstra PT, Mdende N, Mvilli E, van Lieshout L, Hilt ST, Corstjens PLAM, Yazdanbakhsh M, Msuya SE, van Dam GJ. Current status of schistosomiasis in school-aged children in Mwanga district, Tanzania: Impact of two decades of annual mass drug administration programme. *Parasitology*. 2024. DOI: 10.1017/S0031182024001045.

van Dorst MMAR*, **Pyuza JJ***, Nkurununngi G, Kullaya VI, Smits HH, Hogendoorn PCW, Wammes LJ, Everts B, Elliott AM, Jochems SP, Yazdanbakhsh M. Immunological factors linked to geographical variation in vaccine responses. *Nature Reviews Immunology*. 2024. DOI: 10.1038/s41577-023-00941-2.

Pyuza JJ*, van Dorst MAR*, Stam K, Wammes LJ, König M, Kullaya VI, Kruize Y, Huisman W, Andongolile N, Ngowi A, Shao ER, Mremi A, Hogendoorn PCW, Msuya SE, Jochems SP, de Steenhuijsen Piters WAA, Yazdanbakhsh M. Lifestyle score is associated with cellular immune profiles in healthy Tanzanian adults. *Brain, Behavior, and Immunity – Health*. 2024. DOI: 10.1016/j.bbih.2024.100863.

Pyuza JJ*, van Dorst MMAR, Barnett D, Stam K, Manurung M, Wammes LJ, König M, Kruize Y, Andongolile N, Ngowi A, Shao ER, Kullaya VI, Mremi A, Hogendoorn PCW, Msuya SE, Jochems SP, Penders J, Yazdanbakhsh M, de Steenhuijsen Piters WAA. Tanzanian gut microbiota profiles linked to high but rapidly waning yellow fever antibody titers. *NPJ Biofilms and Microbiomes*. 2025. DOI: 10.1038/s41522-025-00687-w.

***Authors contributed equally.**

Other publications not included in this thesis

Jahanpour O, **Pyuza JJ**, Ntiyakunze EO, Mremi A, Shao ER. ABO and Rhesus blood group distribution and frequency among blood donors at Kilimanjaro Christian Medical Center, Moshi, Tanzania. *BMC Research Notes*. 2017. DOI: 10.1186/s13104-017-3037-3.

Pyuza JJ, Andongolile AA, Issangya CE, Msuya D, Yahaya JJ, Shao ER, Mremi AR. Missed opportunity of deworming a Maasai boy from nomadic family leading to life-threatening intestinal obstruction. *Journal of Surgical Case Reports*. 2020. DOI: 10.1093/jscr/rjaa096.

Lema G, Mremi A, Amsi P, **Pyuza JJ**, Alloyce JP, Mchome B, et al. Placental pathology and maternal factors associated with stillbirth: An institutional-based case-control study in Northern Tanzania. *PLoS ONE*. 2020. DOI: 10.1371/journal.pone.0243455.

Pyuza JJ, Shao ER, Bosco K, Lodhia J, Mremi A. An incidental finding of duodenal GIST in a patient with penetrating abdominal trauma: A case report. *International Journal of Surgery Case Reports*. 2021. DOI: 10.1016/j.ijscr.2021.106263.

Suleman M, **Pyuza JJ**, Sadiq A, Lodhia J. Aortic aneurysm: An uncommon cause of dysphagia. *SAGE Open Medical Case Reports*. 2022. DOI: 10.1177/2050313X221135602.

Pallangyo A, **Pyuza JJ**, Nkya G, Amsi P, Andongolile A, Makata AM, Mremi A. Ventricular silent rupture leading to sudden death: Navigating diagnostic challenges in a resource-constrained setting. *Clinical Case Reports*. 2024. DOI: 10.1002/ccr3.8439.

Shirima FL, Keus A, Mmbaga BT, Hooper SB, Mchome B, **Pyuza JJ**, van den Akker T, Te Pas AB. Knee-to-chest flexion manoeuvre to reduce respiratory distress after planned caesarean birth: A feasibility study. *Archives of Disease in Childhood – Fetal and Neonatal Edition*. 2024. DOI: 10.1136/archdischild-2023-326640.

Shao ER, Mboya IB, Gunda DW, Ruhangisa FG, Temu EM, Nkwama ML, **Pyuza JJ**, Kilonzo KG, Lyamuya FS, Maro VP. Seroprevalence of hepatitis B virus infection and associated factors among healthcare workers in northern Tanzania. *BMC Infectious Diseases*. 2018. DOI: 10.1186/s12879-018-3376-2.