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

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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.

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