Overlapping patterns and unique differences: a study into immunological variation within and between populations
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Chapter 1

General Introduction

Adapted from: Immunological factors linked to geographical variation in vaccine responses

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INTRODUCTION

Urbanization is the gradual migration from rural areas to cities and encompasses the transition of previously rural areas towards becoming more urban, along with adoption of the urban lifestyle [1, 2]. Urbanization is on the rise globally with 30% of the global population living in cities in the 1950s to more than 55% today [1, 3]. Currently 80% of people in high-income countries (HICs) live in urbanized areas, while on the African continent this is only 43% [1, 3]. However, low- and middle income countries (LMICs) are catching up rapidly, with an expected increase in total urban population of 57% in Africa and 23% in Asia between 2018 and 2030 [1, 3, 4]. As a result, by 2050 almost 6.5 billion people are expected in to live in urban environments worldwide, 50% of which in cities located in LMICs [5, 6].

Socioeconomic and health inequality in urban areas

Although living in urban areas usually comes with higher income and greater access to essential services, urbanization in LMICS also gives rise to great socioeconomic inequalities that threaten wellbeing and health. As urbanization in LMICs is often rapid and unplanned, urbanization leads to increased levels of pollution, suboptimal or even lack of infrastructure with significant proportion of urban migrants living in slums [7]. Thus, on average, living in a city is more advantageous compared to rural living, but urbanization in LMICs also exacerbates socioeconomic inequalities. Therefore within a single urban center large differences can be observed between individuals from low compared to high socioeconomic status (SES), a combined measure of education, income and type of occupation that indicates an individual's position in society [8, 9]. The socioeconomic inequality greatly affects the wellbeing and health of those living in cities as evidenced by a recent UNICEF report [10]. This report emphasizes large differences in child mortality between those most and least wealthy living in an urban environment. Also, the prevalence of stunting in children was about four times higher in the least affluent compared to the wealthiest urban quartile in many of the LMICs studied. Hence, both geographical location (urban or rural residence) and factors differing within cities or neighborhoods, such as SES, can have great impact on wellbeing and health.

Lifestyle and environmental changes upon urbanization

Urbanization is accompanied by significant changes in lifestyle, including dietary habits, levels of physical activity and exposure to farming environments and pathogens. This shift is commonly referred to as the 'nutritional transition' and describes the changes in physical activity and dietary patterns [11]. Common dietary changes include a transition from the consumption of traditional staple foods rich in fibers, towards more processed foods that are rich in sugars, salt and fat [12, 13]. Moreover, a more sedentary lifestyle is seen in urban dwellers, especially those belonging to high SES, while in rural areas physical labor is often needed to provide food and income [14]. Migration from rural to urban areas also decreases the exposure to agricultural environments, resulting in reduced exposure to microorganisms and parasites such as soil-transmitted helminths [15]. As diet, lifestyle and pathogen exposure are all formative for the microbiome, large differences in the microbiome composition have been observed between urban and rural areas and a more diverse gut microbiomes have been observed in rural and low SES urban individuals compared to (high SES) urban subjects [16-19]. Taken together, urbanization is accompanied by changes in lifestyle that result in differential exposure to environmental factors such as food and microbes, which can impact the microbiome and either directly or through the microbiome affect an individual's health.

Implications of urbanization on health outcomes

By altering all aforementioned factors, urbanization has had and still has strong implications for disease epidemiology and other health related outcomes, such as responses to vaccination. A steep rise in the prevalence of non-communicable diseases (NCDs) such as cardiovascular, inflammatory and allergic diseases, has been observed in the past decades and urbanization has been shown to be a large contributor to this increase [20]. For example, the prevalence of allergic diseases, such as asthma, has increased greatly over the past 50 years, and urban residence has been associated with higher prevalence of asthma [21, 22]. The so-called Old Friends hypothesis may explain the increase in NCDs (23). This hypothesis postulates that the recent increase in NCDs in urbanized societies arises from a failure of the regulation of the immune system. The inadequate regulation of the immune system is thought to result from reduced exposure to microorganisms and parasites with which humans co-evolved, the so called 'Old Friends'. Exposure to these microbes educates the immune system to respond appropriately to harmless stimuli, thereby preventing aberrant immune responses to allergens, self-antigens and gut contents [23, 24]. Although regulation of the immune response is essential to prevent chronic inflammatory diseases, immune regulation can also hamper the response to vaccine antigens, resulting in vaccine hyporesponsiveness. This is evidenced by reduced responses to vaccine antigens in populations living in areas endemic for parasitic helminths that are able to induce strong immune regulatory responses, which have co-evolved with their host [25, 26]. Consequently, large variations in the efficacy of several vaccines have been observed between LMICs and HICs [27-29]. For example, the *Plasmodium* *falciparum* sporozoite (PfSPZ) vaccine protected 92.3% (12/13) of American volunteers against malaria, whereas in Tanzania it only protected 20% (4/20) of the recipients [27]. Vaccine response variation has also been observed within countries, as evidenced by studies comparing vaccine responses among semi-urban and rural Gabonese children [30, 31]. Following a tetanus booster vaccination, rural children demonstrated reduced levels of tetanus specific Interferon-gamma (IFN-γ), a pro-inflammatory cytokine, and skewing towards a more regulated and anti-inflammatory immune profile [31]. In summary, the immune system is for an important part shaped by environmental and lifestyle factors, which greatly determines the response to immune perturbations such as vaccines and allergens or autoantigens.

Unraveling immunological mechanisms

Understanding the immunological mechanisms underlying observed differences in health will help to design interventions to optimize immune response and health in certain populations. For example, studies into the immunological mechanisms underlying altered vaccine responses have revealed similarities between cancer and vaccine hyporesponsiveness. As a result, immunotherapy previously developed for cancer treatment has now been explored in the context of vaccination and has been found to boost the vaccine response *in vitro* [32]. Therefore, studies into immune variation and vaccine responses in populations originating from different areas and urban settings are of great importance and may open up new possibilities to overcome vaccine hyporesponsiveness and thereby improving health.

Immunological differences between countries

Studies comparing populations from LMICs and HICs reveal differences in the immune system that are associated with altered response to stimuli such as vaccines. Although some exposure to microbes is beneficial for the developing immune system, continuous exposure to a high immunostimulatory environment can wear out the immune system. Prolonged activation of the immune system can result in immune exhaustion and/or senescence, a state in which the immune response to new antigens is blunted [33, 34]. A study examining the immune system of Bangladeshi and American children, showed that by 2-3 years of age Bangladeshi children already demonstrated a high degree of immune activation, comparable to that observed in American adults [33]. Moreover, the immune system of the Bangladeshi children was much less able to respond to antigen stimulation [33]. Heightened immune activation is also observed in Ugandan compared to Swiss adults [34]. The baseline immune profile of Ugandans was characterized by exhausted and activated natural killer cells (NK cells), differentiated T and B cell subsets and pro-inflammatory monocytes.

Upon vaccination with the yellow fever (YF) vaccine, responses were significantly lower in Ugandan compared to Swiss volunteers. Moreover, the neutralizing antibody levels negatively correlated with the activation of cells cluster of differentiation 8 positive (CD8+) T cells, B cells and pro-inflammatory monocytes at baseline indicating that heightened activation at baseline is detrimental for the YF vaccine response [34]. This aligns with a study evaluating the response to a novel Ebola vaccine, which reported reduced vaccine responses in Senegal compared to the United Kingdom (UK) and showed that the antibody responses were negatively correlated with expansion of senescent T cells [35]. Higher frequency of these cells were observed in individuals that were positive for cytomegalovirus (CMV) Immunoglobulin G (IgG) and seropositivity for CMV was found to be higher in Senegal compared to the UK, implying a greater infection pressure in Senegal [35]. Thus, studies comparing the immune system of individuals from HICs and LMICs give important clues on how the immune system functions and what immunological mechanisms underly reduced vaccine responses observed in some of these populations.

Urban-rural differences in the immune system

Studies comparing the immune system between countries are insightful, however, are often confounded by between-country differences such as ethnicity, therefore studying immune variation within a country is important. Recently, differences in the immune system of urban and rural populations within a single country have been given more attention and multi-omics approaches have been employed to capture these differences in detail [36, 37]. A study comparing urban and rural Tanzanians revealed that urban individuals had a more pro-inflammatory immune phenotype both at the transcriptional and proteome level [36]. They found that the transcriptome of urban individuals was enriched for the IFN-α and IFN-γ response pathways that play a critical role in the initial pro-inflammatory response to pathogens. Upon stimulation with different bacterial and fungal antigens as well as Toll-like receptor (TLR) agonists *ex vivo*, higher concentrations of pro-inflammatory cytokines (IFN-γ, tumor necrosis factor (TNF) and interleukin 6 (IL-6)) were observed in urban living participants whereas in the rural population, the production of the anti-inflammatory cytokine, IL-10, was higher [36]. A multi-omics analysis of the immune profiles of rural and urban Senegalese and Dutch subjects revealed a continuous trajectory of immune variation along the rural-urban gradient that is driven by both innate and adaptive immune features driving this variation [37]. Rural individuals had higher frequencies of CD11c+ B cells producing pro-inflammatory cytokines and reduced levels of IgG Fc galactosylation, indicating an increased inflammatory state. Moreover, in the rural the NK cell compartment was more differentiated and showed a reduced responsiveness to the Monophosphoryl Lipid-A (MPL), a component of the RTS,S/AS01 malaria vaccine that interacts with TLR-4 [37]. Finally, within the CD4+ T cell compartment, rural individuals showed an enrichment for CD161+ CD4+ T cells with enhanced pro-inflammatory cytokine production capacity, a subset previously also found to be enriched in Gabonese compared to Europeans [37, 38]. Taken together, these studies show that the immune system of rural and urban individuals differ, however, their impact on *in vivo* responses remains speculative, thereby limiting our understanding of the implications of such immunological differences.

SCOPE OF THIS THESIS

Comparing the immune system between different countries has provided us with important insights, as illustrated by previous studies that have described differences between urban and rural populations. However, within these populations a significant degree of immune variation remains unexplained. It has become evident that even within a single urban area a great contrast can exist between high and low SES, however, studies comparing the immune system of individuals with different socioeconomic backgrounds are rare. Moreover, the implications of the immunological differences observed between populations or settings warrants further exploration, as most studies evaluating vaccine responses compare the response between countries rather than within. For these reasons, in this thesis the immune system and vaccine response of rural and urban populations with varying lifestyles and different socioeconomic backgrounds have been studied. Insight in the underlying immunological mechanisms will help to design interventions that are needed to reverse alterations of the immune system that negatively impact health outcomes.

Study populations of this thesis

To capture immune variation among populations with varying levels of urbanization, socioeconomic backgrounds and lifestyles, several field studies have been carried out for this thesis (**Figure 1**). To examine the effect of SES on the immune system, several immune parameters were studied in a population of school-aged children from different socioeconomic backgrounds in Makassar, Indonesia. Makassar is the capital of the Indonesian island of Sulawesi and is one of the four main cities in Indonesia. Makassar has been rapidly expanding due to urbanization, resulting in an estimated population of 1.5 million with large differences in SES [39]. This provides opportunities to assess the impact of SES on the immune system within a single geographical location. In order to further examine differences in immune profiles between populations and to investigate the implications of such differences for response to vaccines, we initiated a study in Northern

Tanzania. This study was called the CapTan study, named after the aim of the study; **Cap**turing variation in the immune system and vaccine response between urban and rural populations in **Tan**zania. This study comprised of a cross-sectional pilot study and a longitudinal study in which participants were vaccinated with the YF vaccine and followed up for six months. For the pilot study, healthy Tanzanians were included from four different areas with different levels of urbanization. The two urban areas were Arusha and Moshi city (referred to as Urban Moshi) and rural Moshi and Mwanga district were selected as the rural areas. Thereafter, urban Moshi and rural Moshi were selected for the longitudinal phase of the study in which participants were randomly assigned into a vaccine group (receiving the YF vaccine) and a control group. All participants were followed up for six months and clinical samples were taken at six different timepoints to study the course of vaccine responses.

Thesis outline

This thesis is divided into seven chapters, each addressing different aspects of the immune system. Chapter 2, 3 and 4 describe the studies performed in Makassar. The current chapter is followed by Chapter 2, in which a total of 945 children with varying socioeconomic background, attending five primary schools all located in Makassar, were studied. In these children the helminth infection status, (growth) hormone levels and Th2 immune markers were measured and their relationship with nutritional status was explored. In Chapter 3, in a smaller subset of children from high and low SES backgrounds, the local immune response in the nasopharyngeal cavity was studied. The density and carriage of four potentially pathogenic bacteria and the level of a wide range of cytokines was determined in the nasal cavity. By comparing the cytokine levels in relation to bacterial colonization, the effect of SES on the local immune response was investigated. In Chapter 4, we profiled the immune cells in finger-prick samples of high and low SES children and studied their characteristics in detail by using mass cytometry. Together, these chapters examine the effect of SES on the composition and the functionality of the immune system. Chapter 5 and 6 report on the CapTan study in Tanzania. In Chapter 5, we examined the cellular immune profiles of 100 participants that were included as part of the cross-sectional pilot study and originated from two urban and two rural areas. Across and within the regions, a substantial degree of variation was observed, which allowed us to study both the effect of location and lifestyle on the cellular immune profile. In Chapter 6, the composition and functionality of the innate immune system at baseline was examined of rural and urban Tanzanians and Europeans vaccinated with YF. To obtain a better understanding of the immunological mechanisms underlying the response to YF vaccination, the innate immune profiles at baseline of high and low responders were compared. Finally, Chapter 7 summarizes the main findings in this thesis and provides directions for future research.

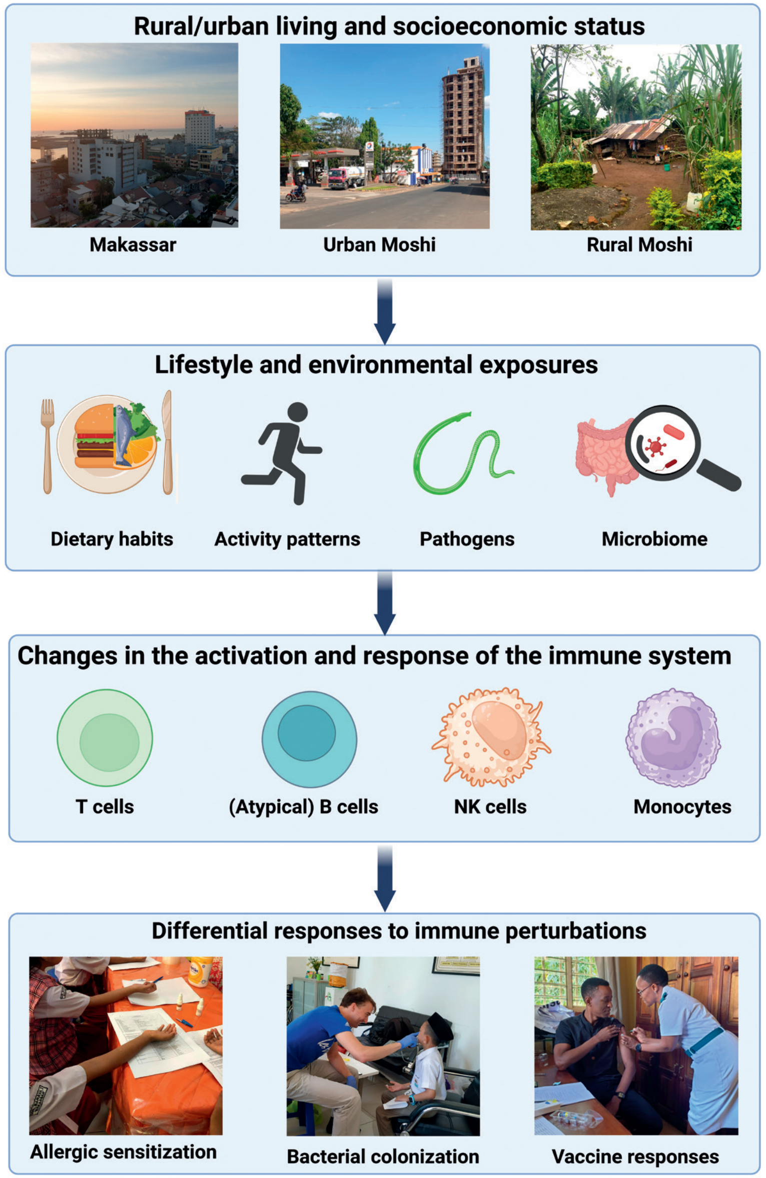

Figure 1. Relationship between geographical location, exposure to environmental factors, changes in the level of activation of and the cytokine responses by innate and adaptive immune subsets and response to immune perturbations that are considered and/or studied in this thesis. *Created in https://BioRender.com*

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