Innate immune response and regulation of human life-histories under adverse conditions

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CHAPTER 1

GENERAL INTRODUCTION

INTRODUCTION

For most of man's history, one of the main threats to survival was infection. It is not surprising that a constant challenge of pathogens resulted in natural selection for adequate defense mechanisms to resist fatal infection[1,2]. Footprints are reflected by many genetic adaptations that associate with higher resistance to infection[3], many of them occurring in the innate immune response[4,5]. This first line of defense against invading pathogens includes recognition and a fast, non-specific response mediated by pro- and anti-inflammatory cytokines. Pro-inflammatory cytokines activate and recruit phagocytic and other effector cells[6] to prevent more extensive infection or dissemination. This response is effective but it can be fatal in excess[7-12]. Anti-inflammatory cytokines are produced as a self-limiting response to prevent damage[13-19]. It has been shown that innate cytokine production and the regulation of pro- and anti-inflammatory cytokine production is under tight genetic control[20,21]. It has also been found that genetic tendency to generate a strong pro-inflammatory response increases survival chance upon infection[21-25]. Therefore it has been hypothesized that in an environment with high infectious burden, early survival is critically reliant on a pro-inflammatory immune response[26,27].

In the natural environment of early hominids, fitness was shaped to live up to the reproductive period up to a maximum of 40 or 50 years[28]. It was only recently that vaccinations, improved healthcare and hygiene have resulted in a decline of mortality in a large part of the world[29]. This 'epidemiological transition' allowed us to age beyond a period where natural selection for beneficial traits could have operated[30-34]. In this ageing process we see a larger incidence of diseases like atherosclerosis, cardiovascular disease, Alzheimer's, cancer or diabetes[35,36]. Interestingly, these degenerative diseases are all related to damage due to chronic inflammation[37]. So, whereas a robust inflammatory response was beneficial for early in life, now we see that later in life sustained inflammation can lead to pathological conditions[38]. Therefore, it can be argued that these age-related diseases are just the other side of the coin: a price we pay to survive in an infectious environment[39]. In other words, it is very likely that these age-related pathologies can be inferred from genetic adaptations that enabled survival in a harsh environment: a pro-inflammatory response[27,38,40-44].

This makes us wonder how the inflammatory host response has been shaped in man's natural environment. Due to infectious pressure on the human genome our fitness has been optimized for an environment where pathogens are constantly provoking the immune response. In order to understand how fitness is optimized with regard to the inflammatory response, in this thesis we study the characteristics of this response in a population living under adverse conditions.

AIM OF THIS THESIS

The general objective of this thesis is to study the characteristics of the inflammatory response in its original context. More specifically several aims can be distinguished. It has been postulated that survival under adverse conditions is largely dependent on a pro-inflammatory innate immune response[26]. Therefore, the first aim of this thesis is to find evidence for selection for a pro-inflammatory responsiveness under adverse conditions. Human survival under adverse conditions is not only dependent on protection for pathogens, but also on reproduction, two traits in which the inflammatory response plays a major role[45]. The second aim is to find evidence that the inflammatory response plays a role in fertility. It is very likely that environmental demands and challenges have shaped this inflammatory response. Therefore the third aim is to study how environmental exposure influences selection for inflammatory response patterns. These objectives have to be studied in an adverse setting where infections are still the major killer. For that reason all studies in this thesis have been performed in a population living in rural Ghana.

A SHORT INTRODUCTION TO INNATE IMMUNE REGULATION

The innate immune response always needs to be prepared for the unknown, so upon infection a non-specific and strong response will be generated to kill a pathogen. For broad identification of the type of pathogens, the host expresses Pattern Recognition Receptors (PRRs)[46]. Toll-like receptors (TLRs) are important cell-bound PRRs. Up-to-date 10 TLRs have been discovered in humans[47]. The most important are the TLR4, a receptor for the eg. Gram negative bacterial endotoxin lipopolysaccharide (LPS)[48], and the TLR2, a re-

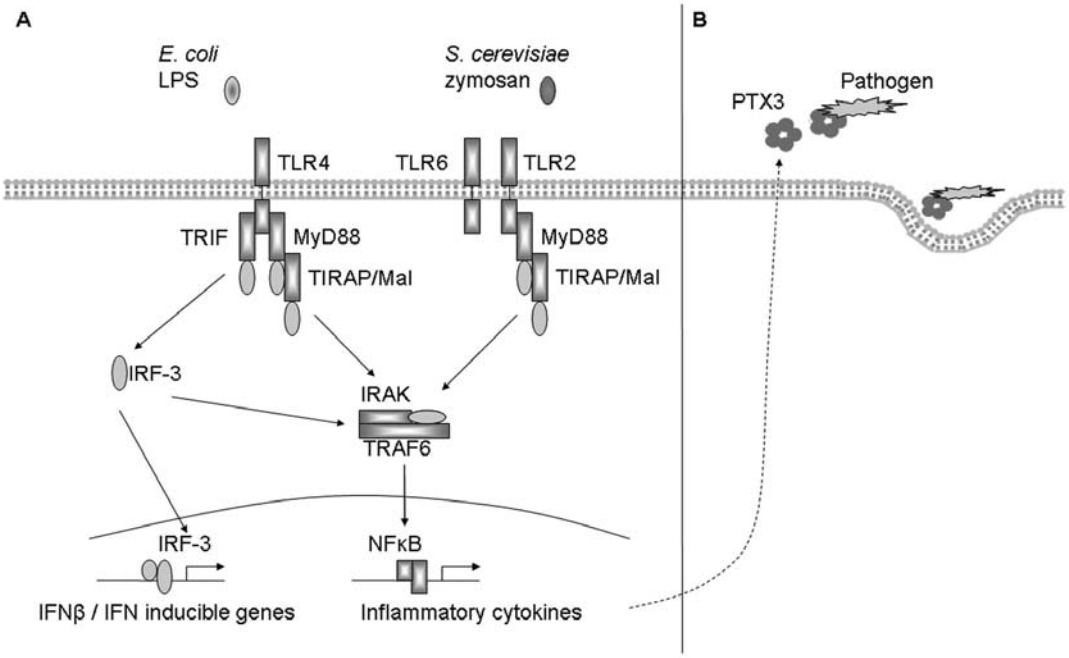

Figure 1. **Innate immune regulation.** A. Toll-like receptors (TLRs) are important sensors for infections, activating innate immune responses. Two important TLRs are TLR4 that binds eg. *E. coli* LPS, and TLR2 that binds eg. *S. cerevisiae* zymosan in costimulation with TLR6. TLR stimulation regulates induction of cytokines via NFκB[46]. B. Inflammatory responses upregulate PTX3 production. PTX3 binds to pathogens eg. yeasts and increases their phagocytosis. PTX3 further initiates complement activation, TLR expression and stimulation of effector cells[55].

ceptor for mostly Gram-positive bacterial peptidoglycans[49] or fungal zymosan[50]. In addition to these two TLRs, also PTX3 is studied in this thesis, which is an important soluble PRR for mainly fungal and viral particles[51]. After recognition the NFκB pathway is induced, resulting in production of downstream pro-inflammatory cytokines, like TNFα[52,53]. Pro-inflammatory cytokines induce further signaling to activate immune cascades and recruit effector cells as neutrophils and macrophages to the affected site (**Figure 1**). A self-limiting mechanism of this response is provided by anti-inflammatory cytokines, like IL10, that downregulate macrophages and control the formation of pro-inflammatory cytokines[54].

STUDY POPULATION

The study described in this thesis was conducted in the Garu-Tempane district of the Upper East Region of Ghana. The population under study counts circa 21.000 people that live in several villages south of the district-municipality Garu. This remote area, close to Togo and Burkina Faso, is enclosed by several natural borders as the Gambaga Scarp plateau and the White Volta river. The area measures approximately 375km^2 (**Figure 2**).

In that region, there is a semi-Saharan climate with an average annual temperature of 32ºC and one rainy season per year from June to August. In the dry season, from December to February, the harmattan - a dusty wind blowing from the Sahara - dries up all land and vegetation. During this time, temperatures raise over 40ºC in daytime and below 15ºC during night. There are several ethnic groups, mainly Bimoba (63%), and Kusasi(27%), two tribes that live in the Upper East Region of Ghana, Togo and Burkina Faso[56]. In contrast to the others tribes that are mostly farmers, the Fulani's (2%) are a pastoralist group and live scattered in the area. This nomadic tribe is present over a large area of Central and West-Africa[57]. Two other minorities are Busanga's (2%) and Mamprusi's (1%).

People live in compounds, which is a formation of several clay huts, with roofs of grass, surrounded by a clay wall (**Figure 3**). In this compound lives one family, consisting of a landlord with his wives, their children and occasionally his mother. Cattle are placed in a hut at the outer side of the compound. Compounds are surrounded by crops as millet, corn, beans, peanuts and cotton. As there is only one fertile season per year, food availability will be poor just before the raining period.

The population is poor and the estimated gross domestic product per capita is less than $100[58,59]. Mortality rates are high, especially maternal and child mortality. The main cause of death among children is malaria, which is endemic in this region; in a survey we have found that under 30 years of age, 100% are carrier of *P. falciparum*. Other endemic diseases are typhoid fever, diarrheal diseases and intestinal helminth infections. In the total population, for example 30% is carrier of hookworm, and 60% is carrier of *G. lamblia*.

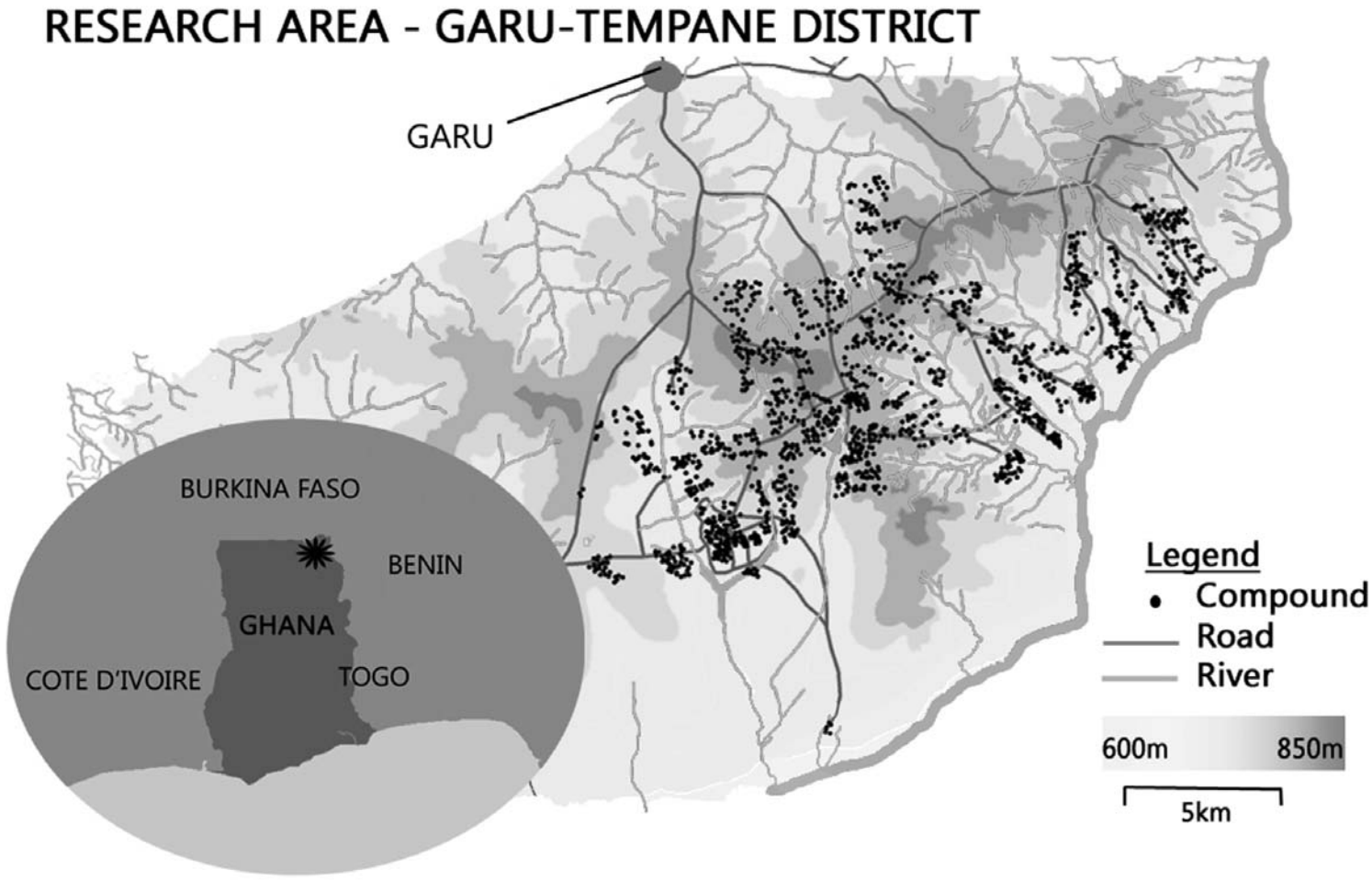

Figure 2. Overview of the research area in Garu-Tempane District, located in the Upper East Region of Ghana. Close to the district municipality Garu, several small villages were selected to be included. Compounds are indicated in black. Geographic data were obtained from CERGIS center of the Legon University, Accra.

Despite the very remoteness and the apparent underdevelopment of the major part of this area, some progress is taking place. Boreholes have been placed in the area[60,61], starting in the 1980s, now giving access to clean drinking water for 81% of the population. Mass vaccination against polio, measles, whooping cough, diphtheria, tetanus, tuberculosis and typhoid has been introduced in the early 1990s as a national initiative. Full vaccination has been reported to be around 50%[62]. Also mass-treatments have been performed for control of helminth-infections[63-68]. Although in the area there is no hospital - the closest hospitals are in Binde, Bawku or Nalerigu with an average distance of 30 km - there are some health posts with nurses situated in the area. Garu has a larger health clinic that recently got a doctor and an ambulance. As of 2006 the National Health Insurance Scheme has been implemented in this area, allowing free healthcare. In addition, some compounds have iron roofing (61%) or electricity (15%) and motorbikes, mobile phones and networks have made their entrance to the region. Also developed is the investment in schooling; whereas almost 100% of the adults are illiterate, in a survey we have estimated that around 40% of the children are now going to school. All developments have contributed to declined mortality rates in recent years[69,70].

STUDY DESIGN

The exploration of the study area started with a project on controlling Oesophagostomum-infections in 2001, by the Department of Parasitology. A database was set up that included name, sex, estimated age, tribe and address of all individuals in the defined research area. Compounds were registered by Global Position System (GPS). Since 2003 the department of Gerontology and Geriatrics has been studying life-history regulation. The study of the present thesis started in 2005. More recently mortality has been studied from a social evolutionary perspective.

We have followed the population in the Garu-Tempane district for survival using an annual update for migration, births and mortality. Each year mucous swabs were obtained from the newborns for DNA extraction in cooperation with the department of Molecular Epidemiology. Besides newborns also a group of women and elderly people were included in DNA analyses.

Figure 3. **Impression of the research population.** Above: compounds in wet and in dry season. Compounds consists of a formation of clay huts, with roofs of grass, surrounded by a wall. The interior of the compound including a cooking place is shown on the right. Below: typical Bimoba family living in one compound, here depicted is the landlord, his mother his wives and children.

In 2003, the fertility history – measured as the amount of children conceived in their lives at the time of questioning - was obtained from women in the area[59]. In 2005 whole-blood stimulation assays were performed from a sub-sample, including women, children and elderly men and women, in cooperation with the department of Clinical Chemistry. Due to contamination of the previous series, this was repeated in 2006. In 2007, data was obtained on socioeconomic status and access to clean drinking water. In 2008, blood samples were obtained for tests on malaria infection.

The field-station, from where all fieldwork was coordinated, was in Garu. Also the fieldworkers originated from Garu or one of the villages in the research area. The office in Garu also included a small laboratory with limited facilities and was equipped for performing whole blood assays only.

MEASURING INNATE IMMUNE RESPONSES: THE WHOLE BLOOD CYTOKINE STIMULATION ASSAY

The whole blood assay has been developed to measure immune responses *ex vivo*[71]. In this assay, infection is simulated via TLR stimulation, under conditions that resemble that of the human body, i.e. a humid and CO_2 rich atmosphere of 37ºC. Often *E. coli* lipopolysaccharide (LPS) is used as a TLR4 agonist[48]. In this study we have incubated the whole blood assay for 24 hours, where the cytokines produced represent that of the innate immune response. Cytokines formed during incubation are quantified by Enzyme Linked Immunosorbent Assay (ELISA), a technique that uses fluorescent labeled antibodies towards human cytokines to estimate their concentration based on optic density.

GENETIC VARIATION OF CANDIDATE GENES

Genetic variation between individuals can be studied by single nucleotide polymorphisms (SNPs). These are DNA sequence variations that occur as a result of a single nucleotide change and can alter the encoding protein in shape or transcription. Due to inheritance, they occur throughout the population and might explain for the difference in phenotypes. By association studies we investigate patterns of variations between individuals with different phenotypes on a population scale. In this thesis most of the genetic variation of the studied genes of interest is captured by tagging SNPs, a method to select a minimum number of SNPs to incorporate as much as possible of the genetic variation present in the gene under study. By doing so, we try to understand the role of genetic variation in the development of certain traits, like fertility and survival.

OUTLINE OF THE THESIS

In **Chapter 2**, the general framework of the Ghana study is explained. In this chapter we elaborate on the role of the innate immune response in the trade-off between fertility and maintenance for human populations living under adverse conditions. We hypothesize that under infectious pressure pro-inflammatory responses are favored and evaluate what the consequen-

ces are of this evolutionary programming for populations living under affluent conditions.

In **Chapter 3** we assess how to measure innate tendency of immune activation under field-conditions, where materials and methods are limited. Inspired by an earlier series of contaminated whole-blood assays, we design an assay that is more suitable in the field. This assay with several TLR-agonists reflects general immune responses more than the former pathogen-specific assay. Furthermore, by repeated measurements, we compare the validity of this assay to that performed under ideal conditions in the Netherlands.

In **Chapter 4** we assess whether there is evidence for selective survival of a pro-inflammatory innate immune profile in the Ghanaian population. Therefore we compare age-related cytokine production cross-sectionally in two populations living under adverse conditions in Ghana and under affluent conditions in the Netherlands.

As TLR2 and TLR4 are important receptors for pathogen recognition and activation of the innate immune response, in **Chapter 5** we study their genetic variation. We assess whether genetic variation in the *TLR4* and *TLR2* genes is associated with cytokine production, malaria susceptibility and selective survival.

Another important pathogen receptor is PTX3 that has, besides pathogen recognition, an important role in female fertility. In **Chapter 6** we test if genetic variation in the *PTX3* gene is associated with PTX3 production capacity, fertility and survival.

Earlier we hypothesized that there might be selection for pro-inflammatory responsiveness. This we test in **Chapter 7**. We assess whether genetic variation in the *IL10* gene is associated with cytokine production and survival. Furthermore we test whether there is evidence that environmental conditions play a role in the survival benefit of these genetic variants.

In **Chapter 8** all results are discussed in scope of life-history regulation and improved environmental conditions. Here also further research directions are given.

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