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Parasitic infections and immune function: Effect of helminth infections in a malaria endemic area

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ABSTRACT

According to the hygiene hypothesis, reduced exposure to infections could explain the rise of atopic diseases in high-income countries. Helminths are hypothesised to alter the host's immune response in order to avoid elimination and, as a consequence, also reduce the host responsiveness to potential allergens. To elucidate the effect of current helminth infections on immune responsiveness in humans, we measured cytokine production in a rural Ghanaian population in an area with multiple endemic parasites including malaria, intestinal helminths and protozoa. Multiplex real-time PCR in stool samples was used for the detection of four gastrointestinal helminths, of which only *Necator americanus* was commonly present. A similar assay was used to test for *Giardia lamblia* in stool samples and malaria infection in venous blood samples. Levels of the cytokines interleukin (IL)-10, tumour necrosis factor (TNF)-α, IL-17, IL-6, IL-13, and interferon (IFN)-γ were determined in whole-blood samples *ex vivo*-stimulated either with lipopolysaccharide (LPS) and zymosan (for innate cytokine production) or the T-cell mitogen phytohaemagglutinin (PHA). There were no significant differences in either innate or PHA-stimulated cytokine production dependent on current *N. americanus* infection. *Plasmodium falciparum* malarial infection was associated with a pro-inflammatory response indicated by increased innate production of TNF-α, IL-17 and IL-6. There was no clear pattern in cytokine responses dependent on *G. lamblia*-infection. In conclusion, in this rural Ghanaian population current *N. americanus* infections are not associated with altered immune function, while infection with *P. falciparum* is associated with pro-inflammatory innate immune responses.

Introduction

According to the hygiene hypothesis, reduced exposure to infections could explain the rise of atopic diseases in high-income countries (Strachan 1989). Epidemiological studies have focused in particular on the possible inverse relationship between helminth infections and allergic disease. Though the evidence from these studies does not provide general consensus on the association between helminth infection and allergic disease, it indicates that chronic and intense helminth infections are negatively associated with allergic reactivity (Smits et al. 2010). Helminths are hypothesised to alter the immune response of the host in order to avoid elimination and, as a consequence, also reduce the host responsiveness to potential allergens (Diaz and Allen 2007).

Both allergic disorders and helminth infections induce strong type 2 responses (Smits et al. 2010), characterised by production of T-helper type 2 (Th2) cytokines (e.g. IL-4, IL-5, and IL-13) (Diaz and Allen 2007; Yazdanbakhsh et al. 2002), tissue eosinophilia and IgE production (Smits et al. 2010; Yazdanbakhsh et al. 2002). The difference between immune responses in these two conditions could be that helminth infections also induce a strong regulatory network in addition to the type 2 response (Yazdanbakhsh et al. 2002). This regulatory response has been proposed to explain the inverse association between helminth infections and allergy, through suppression of not only helminth-specific responses but also of general T-cell responsiveness (Smits et al. 2010). Previously described mechanisms of this regulatory response include regulatory T-cell (Treg) activity (Diaz and Allen 2007; Smits et al. 2010) and the modulation of innate immune cells by helminth products (Smits et al. 2010). Interleukin-10 (IL-10) has been described as a

Abbreviations: Ct, cycle threshold; IFN-γ, interferon-gamma; IL, interleukin; LPS, lipopolysaccharide; PHA, phytohaemagglutinin; PhHV-1, phocin herpes virus 1; Th2, T-helper type 2; TLR, toll-like receptor; TNF-α, tumour necrosis factor-alpha; Treg, regulatory T cell.

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key mediator in the regulatory response. It is produced by innate immune cells, Treg cells and possibly by regulatory B-cells (Smits et al. 2010) and has inhibitory effects on the production of pro-inflammatory cytokines such as TNF-α (Moore et al. 2001). On the cytokine level, we would therefore expect helminth-infected individuals to have a higher IL-10 production and lower levels of pro-inflammatory cytokines than non-infected individuals.

Most previous studies regarding the mechanisms which potentially underlie the hygiene hypothesis have used *in vitro* or murine models. Fewer studies have investigated the effect of an ongoing helminth infection on innate and adaptive immune function under "pre-industrial" conditions, i.e. in a population living in an environment with high infectious pressure (van der Kleij et al. 2004; Everts et al. 2010; Figueiredo et al. 2010; Geiger et al., 2002, 2004; Turner et al. 2008; Meurs et al. 2011). Opportunities to directly study the effects of helminth infections on immune responsiveness in humans living under high infectious pressure are rare but important, as they allow the possibility of studying the effect of helminth infections on the human host in the environment where they co-evolved. We therefore studied the relationship between intestinal helminth infection and cytokine production upon different stimuli in a Ghanaian population living in an area with multiple endemic parasites including malaria and intestinal protozoa.

Materials and methods

Population characteristics

The study was conducted in a rural and impoverished area in Ghana's Upper-East region. The climate is semi-Saharan with a single rainy season from May/June to September/October. The majority of inhabitants of the area are subsistence farmers. They belong to several different tribes, mainly Bimoba and Kusasi. Most live in extended and often polygynous families. The main causes of death in the region are infectious diseases, both in childhood and in adulthood (Ghana Health Service 2004) and availability of medical services is very limited. The research area has been followed annually since 2002 for demographic updates and additional data collection. Mass treatment with albendazole was carried out in part of the area in 2001 and twice in 2002 as part of a study aimed at controlling *Oesophagostomum* infections. This was followed by mass treatment with albendazole and ivermectin of an estimated 72% of eligible subjects in the entire area in 2003 as part of the national Lymphatic Filariasis Elimination programme (Ziem et al. 2006). Albendazole also treats hookworm infections. In 2007, socioeconomic status was defined by a method similar to the Demographic Health Survey (DHS) wealth index (van Bodegom et al. 2009). Drinking source was determined by asking within each district (consisting of several compounds within a village) whether there was access to a borehole or if a well or river was used as a drinking source. Based on the purity of the water sources, drinking source was then classified as fairly safe (from borehole) or unsafe (from river or well).

Ethical considerations

Ethical approval was given by the Ethical Review Committee (ERC) of the Ghana Health Service, the ERC of the Leiden University Medical Center in Leiden, The Netherlands, and by the local chiefs and elders of the research area. Informed consent was obtained from all participants.

Parasitic infection

Stool samples were collected in 2008 from 610 apparently healthy volunteers. The majority of volunteers were primarily selected for a study on the relation between immune responsiveness and fertility (results not published). Therefore mainly women from different age categories of whom the fertility history had been previously collected were selected (May et al. 2010). To determine the presence of intestinal helminth infection, multiplex real-time PCR was performed for *Ancylostoma duodenale*, *Necator americanus*, *Oesophagostomum bifurcum* and *Strongyloides stercoralis*, the common intestinal nematode species of the region. The methods of DNA isolation, PCR amplification and detection and the PCR primers used have previously been described in detail elsewhere (Verweij et al. 2007, 2009). The output of the real-time PCR is a cycle threshold (Ct), which is the amplification cycle in which the fluorescent signal exceeds the background fluorescence. The Ct can be used as a quantitative outcome, with lower values indicative of higher loads of parasitic DNA (Verweij et al. 2007). Phocin herpes virus 1 (PhHV-1) was added to the sample and included in the assay as an internal control, in order to detect inhibition of amplification due to faecal contaminants (Verweij et al. 2007). A similar multiplex real-time PCR was performed on the same stool samples to determine the presence of *Giardia lamblia* (Verweij et al. 2003). A multiplex real-time PCR for the four malaria species *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale and Plasmodium malariae* was performed on venous blood samples also collected in 2008 (the methods of which have been previously described elsewhere (Wiria et al. 2010)).

Cytokine production

From 608 of the participants who provided stool samples, venous blood samples were collected in 2008. Whole-blood samples were incubated with 10 ng/mL *Escherichia coli* lipopolysaccharide (LPS, a TLR4 ligand) and 100 μg/mL of *Saccharomyces cerevisae* zymosan (a TLR2 ligand) for 24 h at 37 °C in a humidified atmosphere containing CO_2 as previously described in more detail elsewhere (May et al. 2009). A similar procedure was performed using 2 μg/mL of the T-cell mitogen phytohaemagglutinin (PHA) and a longer incubation time of 48 h. The supernatant of the centrifuged samples was aliquoted, stored at −20 °C and transported to the Netherlands on dry ice. Concentrations of IL-10, TNF-α, IL-13, IL-17, IL-6 and IFN-γ were measured in the Netherlands by a multiplex assay (Bio-Plex assay, Bio-Rad Laboratories, Veenendaal, The Netherlands).

Large variation in concentrations of the different cytokines in the LPS/zymosan-stimulated multiplex assay resulted in a proportion of measurements higher than the reliable range for TNF-α and IL-6 and measurements lower than the reliable range for IL-13. TNF-α levels were therefore measured again using Enzyme-Linked Immunosorbent Assay (ELISA) according to the manufacturer's protocol (PeliKine Compact™ human TNF-α, Sanquin Reagents). LPS/zymosan-stimulated levels of IL-6 were dichotomised and defined as either within the reliably measurable range (<61,797 pg/ml) or higher (≥61,797 pg/ml). LPS/zymosan-stimulated IL-13 measurements were not included in the results. The intra-assay coefficients of variability (CV) for the multiplex LPS/zymosan-stimulated assay ranged from 4.6% to 8.5% except for IL-6 which had an intra-assay CV of 20.5%. For the PHA-stimulated assay the intra-assay CVs ranged from 6.2% to 7.8%.

Statistical analysis

Cytokine levels were log-transformed (with base 10) because they were not normally distributed and subsequently converted into *Z*-scores. *Z*-scores represent standard deviations from the population mean (*Z*-score = [observed value − sample mean]/sample standard deviation).