



Helminth infection is associated with dampened cytokine responses to viral and bacterial stimulations in Tsimane forager-horticulturalists

India A. Schneider-Crease ^{1,*} Aaron D. Blackwell ² Thomas S. Kraft,³ Melissa Emery Thompson,⁴ Ivan Maldonado Suarez,⁵ Daniel K. Cummings,⁶ Jonathan Stieglitz ⁷ Noah Snyder-Mackler,^{1,8} Michael Gurven ^{3,†} Hillard Kaplan^{6,†} and Benjamin C. Trumble^{1,8,9,†}

¹Center for Evolution and Medicine, Arizona State University, Tempe, AZ, USA; ²Department of Anthropology, Washington State University, Pullman, WA, USA; ³Department of Anthropology, University of California Santa Barbara, Santa Barbara, CA, USA; ⁴Department of Anthropology, University of New Mexico, Albuquerque, NM, USA; ⁵Tsimane Health and Life History Project, San Borja, Bolivia; ⁶Economic Science Institute, Chapman University, Orange, CA, USA; ⁷Institute for Advanced Study in Toulouse, Toulouse, France; ⁸School of Life Sciences, Arizona State University, Tempe, AZ, USA and ⁹School of Human Evolution and Social Change, Arizona State University, Tempe, AZ, USA

*Corresponding author. Center for Evolution and Medicine, Tempe, AZ, USA. Tel: +480 965 9946; Fax: +480 727 4457; E-mail: indiasc@asu.edu

†The last three authors are co-senior authors.

Received 03 May 2021; revised version accepted 19 October 2021

ABSTRACT

Background: Soil-transmitted helminths (STHs) and humans share long co-evolutionary histories over which STHs have evolved strategies to permit their persistence by downregulating host immunity. Understanding the interactions between STHs and other pathogens can inform our understanding of human evolution and contemporary disease patterns.

Methodology: We worked with Tsimane forager-horticulturalists in the Bolivian Amazon, where STHs are prevalent. We tested whether STHs and eosinophil levels—likely indicative of infection in this population—are associated with dampened immune responses to *in vitro* stimulation with H1N1 and lipopolysaccharide (LPS) antigens. Whole blood samples ($n = 179$) were treated with H1N1 vaccine

and LPS and assayed for 13 cytokines (INF- γ , IL-1 β , IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12p70, IL-13, GM-CSF and TNF- α). We evaluated how STHs and eosinophil levels affected cytokine responses and T helper (Th) 1 and Th2-cytokine suite responses to stimulation.

Results: Infection with *Ascaris lumbricoides* was significantly ($P \leq 0.05$) associated with lower response of some cytokines to H1N1 and LPS in women. Eosinophils were significantly negatively associated with some cytokine responses to H1N1 and LPS, with the strongest effects in women, and associated with a reduced Th1- and Th2-cytokine response to H1N1 and LPS in women and men.

Conclusions and implications: Consistent with the ‘old friends’ and hygiene hypotheses, we find that STHs were associated with dampened cytokine responses to certain viral and bacterial antigens. This suggests that STH infections may play an essential role in immune response regulation and that the lack of STH immune priming in industrialized populations may increase the risk of over-reactive immunity.

Lay Summary: Indicators of helminth infection were associated with dampened cytokine immune responses to *in vitro* stimulation with viral and bacterial antigens in Tsimane forager-horticulturalists in the Bolivian Amazon, consistent with the ‘old friends’ and hygiene hypotheses.

KEYWORDS: soil-transmitted helminths; viruses; bacteria; cytokine storms; eosinophilia; hypereosinophilia; immunomodulation; hygiene hypothesis; old friends hypothesis

INTRODUCTION

Infection with soil-transmitted helminths (STHs) can modulate the host immune response and ultimately shape morbidity and mortality associated with viral and bacterial infections [1, 2]. The vast majority of human history occurred in environments characterized by high STH prevalence, which has fundamentally shaped the evolution of human immunity and created a potentially essential role for STHs in regulating immunity in the face of co-infections [3–5]. Indeed, the mismatch between high STH rates over human evolutionary history and their near absence in many contemporary industrialized communities has been linked to immune dysregulation resulting in over-reactive immune function and allergy [5–7], while the presence of STHs in more rural communities has been hypothesized to play a protective role against runaway inflammatory immune responses [4, 5, 8]. This is suggested to be a product of a coevolutionary trajectory that has led to relatively low helminth virulence via parasite-induced manipulation of the host immune system [1, 5]. These relationships are often conceptualized as the ‘hygiene hypothesis’ and the ‘old friends’ hypothesis.

The hygiene hypothesis argues that there is a mismatch between current sanitary urban environments and the conditions in which the majority of human evolution occurred, such that the immune systems of many contemporary urban populations that evolved to cope with high levels of pathogen exposure instead experience low pathogen exposure throughout their lives. This lack of immune stimulation and priming results in dysregulated adult immune function and gives rise to increased rates of allergy and autoimmune infection [5]. The ‘old friends’ hypothesis builds on the hygiene hypothesis, focusing specifically on the co-evolutionary role of macroparasites in priming T-helper type 2 (Th2) immune activation [1]. STHs can catalyze trade-offs between Th1 and Th2 responses that can modulate

immunological responses to other pathogens. The ‘old friends’ hypothesis suggests that the lack of co-evolved parasites and pathogens in contemporary urban environments releases the Th1 response from Th2 modulation or regulation, and that higher rates of autoimmune disorders and allergies in industrialized populations can be attributed to a lack of helminth-induced moderation of reaction to self-antigens and allergens [1, 5, 7]. Lack of STH immune priming may also increase the risk of overreaction to mild or moderate viral and bacterial infections, which has been hypothesized to increase morbidity and mortality associated with conditions such as COVID-19 [4, 8]. Together, both hypotheses center on the idea that changes in pathogen exposure affect immune function in ways that shape disease susceptibility. However, because most medical research takes place in industrialized urban environments, our understanding of potentially deleterious and protective interactions between STHs and immune function is limited.

STHs elicit Th2-polarized immune responses in the simplified Th1/Th2 paradigm [9, 10]. Within this paradigm, naive CD4⁺ cells are expected to differentiate into cells with distinct functions based on antigen presentation [11]. Intracellular parasites (e.g. protozoa, viruses) typically trigger Th1 responses that activate a broadly proinflammatory response (e.g. interferon gamma (IFN- γ), interleukin-2 (IL-2)). Helminths trigger Th2 responses that activate cells to release anti-inflammatory and regulatory cytokines, which mediate the activation of effector mechanisms that include the antibody-based immune response and regulatory T cells [9, 12, 13], inhibit the proinflammatory Th1 response, and modulate antigen reactivity [14–16]. Helminths may thus be powerful modulators of the immune response to subsequent infections.

Among the most devastating outcomes of certain viral and bacterial infections is the induction of a hyperactive cytokine response (‘cytokine storm’) that can cause tissue and organ

damage and is associated with many of the deaths in viral outbreaks, including the current COVID-19 pandemic [8, 17]. Cytokine storms are characterized by an unfettered increase in proinflammatory cytokines [18] and are associated with a greater incidence of severe illness and mortality [17, 19]. Because STHs can inhibit proinflammatory Th1 responses and promote regulatory T cells [16, 20], they may, paradoxically, provide protection against some of the worst outcomes of viral and bacterial infections.

Understanding how STHs affect downstream viral and bacterial infections is particularly crucial for non-industrialized or rural populations with high infection prevalence. Over 12% of the world's population is infected with at least one STH [21] and the spread of highly contagious viral and bacterial infections continues to accelerate via globalization and international travel [22]. Thus, understanding the ways in which STHs affect the immunological response to and outcomes of viral and bacterial infections is particularly exigent.

This study focuses on STHs and immune response in Tsimane forager-horticulturalists of the Bolivian Amazon, who practice a largely non-industrial lifestyle centered around hunting, fishing, gathering and small-scale slash-and-burn farming, and have relatively little interaction with market economies [23–25]. The Tsimane have high STH rates (up to 76% [26, 27]) which is likely linked to the lack of sanitation infrastructure, inconsistent access to footwear, shared space with animals and open defecation practices [23, 28, 29]. We assess the hypothesis that STHs inhibit the cytokine response to viruses and bacteria by dampening the proinflammatory cytokine phenotype using whole blood stimulation with H1N1 vaccine and lipopolysaccharide (LPS) antigens. LPS is a component of gram-negative bacteria cell walls that induces an innate immune response characterized by inflammation in stimulations [30]. The H1N1 vaccine allows for viral stimulation without using live virus in the absence of a completely controlled laboratory setting. At the time of data collection, H1N1 was not known to have spread in the area and thus would represent a novel strain that the participants had not yet encountered.

We evaluate the responses of 13 cytokines and two functional cytokine suites (e.g. pro- and anti-inflammatory) to H1N1 vaccine and LPS as a function of two metrics related to parasitism: the presence of each of the five most common STHs in this population and eosinophil count, a measure of white blood cell activation that is part of the STH immune response. We evaluate these patterns separately in women and men, predicting that metrics of parasitism will be associated with lower proinflammatory immune responses and that women will exhibit stronger proinflammatory responses than men based on higher female immune responsiveness (e.g. increased immune cell

proliferation and activation, upregulation of immune-specific genes) [7, 31, 32]. All predictions are summarized in Table 1.

METHODS

The Tsimane Health and Life History Project

The Tsimane Health and Life History Project (THLHP) has worked with a population of approximately 16 000 Tsimane across ~90 villages since 2002, providing routine medical care and collecting epidemiological and biodemographic data [23]. Medical care is provided to all individuals and informed consent is obtained from individuals, villages and the Tsimane government (Gran Consejo Tsimane). All research is approved by the University of New Mexico and University of California Santa Barbara Institution Research Boards (IRB # 07-157, 15-133). None of the individuals who participated in this study had received anthelmintic treatment (i.e. mebendazole, albendazole) or antibiotics from our medical staff within at least 8.5 months (mean for the 71% of sampled individuals with a record of treatment = 1.4 years) of sample collection beginning in March 2011. To our knowledge, no Tsimane had ever been vaccinated against H1N1 or other viruses at the time of this study.

Biomarker data collection

Biomarker data were collected from 179 Tsimane adults who participated in an antigen stimulation study. This sample included 82 women aged 29–71 (median age: 46.5) and 97 men aged 37–89 (median age: 49). Six women were pregnant at the time of the study (based on back-calculating from next birth). Participants attended the THLHP Clinic in San Borja, Bolivia for routine medical care and biodemographic data collection between March–November 2011. Fasting morning blood (5 ml) was collected into a heparinized vacutainer tube. A manual white blood cell count and five-part differential (including eosinophils) were conducted with a hemocytometer immediately following each blood draw, and fecal samples were collected for parasite identification using fecal smear microscopy or a density separation technique [28].

Helminth infections

We characterized parasite communities by identifying parasite species with direct smear (30% of samples) microscopy [27] as well as Percoll® separation (70% of samples) in fresh fecal samples [2]. We placed all hookworm eggs in a single category because the eggs of hookworm species can be difficult to differentiate morphologically. We combined the results of both

**Table 1.** Predictions, expected cytokine effects, and results

Prediction	Predicted effects	Results (H1N1 vaccine)	Results (LPS)
STH presence/absence associated with lower proinflammatory immune response to H1N1 vaccine and LPS stimulation	↓IFN- γ , IL-2; ↓Th1-suite; women > men	Women: ↓ IL-1 β , IL-2, IL-6, IL-7, IL-10, IL-13, GM-CSF (<i>A. lumbricoides</i> only) Men: none	Women: ↓ IFN- γ and IL-7 (<i>A. lumbricoides</i> only) ↓ Th1-suite Men: ↓ IFN- γ (hookworm sp. only)
Eosinophil count associated with lower proinflammatory immune response to H1N1 vaccine and LPS stimulation	↓ IFN- γ , IL-2; ↓ Th1-suite; women > men	Women: ↓ IFN- γ , IL-1 β , IL-2, IL-4, IL-7, IL-8 ↓ Th1-suite Men: ↓ IFN- γ , IL-2 ↓ Th1-suite	Women: ↓ IFN- γ , IL-4, IL-6, IL-7, IL-8, GM-CSF ↓ Th1-suite Men: ↓ IFN- γ , IL-1 β , IL-6, IL-7, IL-8 ↓ Th1-suite

methods for all analyses, and assessed co-occurrence of parasite species with a Pearson's correlation matrix.

Eosinophils

Microscopic techniques such as direct smears and density separations can produce false negatives based on non-random distribution of eggs in feces and life cycles [33–35]. In addition, 30% of our fecal samples were not processed for quantitative egg counts (those processed with direct smears rather than Percoll separation), precluding estimation of infection intensity across the dataset. We thus performed additional analyses, first on the relationship between parasite infections and eosinophils, which occupy a cardinal role in the helminth immune response [36] and are a common indicator of STHs in clinical settings [37, 38]. We modeled eosinophils (cells/ μ l) as a function of each parasite (presence/absence) for species with >10% prevalence in generalized linear models (GLMs) with age included as a continuous predictor in each model (women/men). We then used eosinophil count as a proxy of parasitism in downstream analyses.

Eosinophils can be associated with allergies or autoimmune disorders in industrialized populations; however, as in many non-industrialized populations [1], virtually no allergies or autoimmune disorders have been identified among the Tsimane [26]. This absence may arise from the regulatory immunophenotypes induced by helminths (indeed, antihelminthic treatment is associated with lower eosinophils in high STH prevalence areas) [39, 40], and suggests that—while we cannot rule out eosinophilic leukemia or other conditions—eosinophils are likely to indicate helminth infection in the Tsimane. We use eosinophil count here as a secondary metric to the presence/absence

of each STH that may capture additional elements of infection, including infection intensity or periods of migratory larval stages characterized by pre-reproductive larvae that do not yet produce eggs.

In vitro antigen stimulation

Aliquots of 100 μ l heparinized whole blood were immediately added to separate round bottom microtiter wells in a sterile 96-well plate. One aliquot received 1 μ g/ml H1N1 vaccine [2009 Monovalent Vaccine (Sanofi Pasteur, Inc. Swiftwater PA 18370)] diluted in RPMI-1640. Another aliquot received 100 μ l of 20 μ g/ml LPS (Sigma cat. L2630) diluted in RPMI-1640, rendering a final concentration of 10 mg/ml LPS. Control aliquots were run in RPMI alone. To prevent contamination, RPMI was supplemented with 100 IU/ml penicillin and 100 μ g/ml streptomycin (Sigma cat. P0781), and any plates with visible growth or control values indicating contamination were eliminated. We enriched CO₂ concentration by sealing plates in an airtight container with a burning candle to deplete O₂ as a field-friendly alternative to a CO₂ incubator [41]. The sealed and treated blood samples were incubated at 37°C for 72 h. Samples were centrifuged and supernatants were frozen in liquid nitrogen, transported on dry ice, and stored at –80°C for up to 2 years (see [42, 43]).

At the Hominoid Reproductive Ecology Laboratory at the University of New Mexico, 13 cytokines (INF- γ , IL-1 β , IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12p70, IL-13, Granulocyte-macrophage colony-stimulating factor (GM-CSF), and Tumor necrosis factor-alpha (TNF- α)) were measured with a Milliplex MAP High-Sensitivity Human Cytokine Panel (HSCYTMAG-60SK-13, Millipore Corp., Billerica, MA, USA) on a Luminex MagPix

(Millipore Corp., Billerica, MA, USA). All quality control specimens were within normal limits.

Statistical analysis

Effect on individual cytokine responses. We assessed the impact of STHs on the response of each cytokine to stimulation with H1N1 and LPS antigens. Assessing women and men separately, we used GLMs to model the log-transformed concentration of each cytokine in either H1N1 or LPS as a function of the presence or absence of each STH. We included body mass index (BMI) and age as covariates in all models, and included a pregnancy covariate for women (0/1) [44]. The small number of pregnant women ($n=6$) precluded a trimester-level analysis [45]. We included only STHs with $>10\%$ prevalence across the sample set.

We assessed the impact of eosinophil count (cells/ μl) on individual cytokine response to stimulation. We used GLMs to model the response of log-transformed cytokines in response to stimulation with H1N1 and LPS by eosinophil count. We included BMI, age, and pregnancy for women, and included total leukocyte count to account for variation in total white blood cells. We assessed collinearity between eosinophil and leukocyte counts by examining the variation inflation factor (VIF) for all models; all VIFs were acceptably low (<2.5). We found no significant interaction between age and eosinophils and thus omitted this interaction from our final models.

We calculated a meta effect score by combining the fixed effects from each model for women and men ('meta' package [46]). Although this approach assumes cytokine independence, it allows for a broad estimation of the cross-cytokine effect of eosinophils and functions as a summary statistic.

We did not consider changes from baseline cytokine levels (unpublished data) because cytokines have short half-lives, ranging from minutes to hours [47]; thus, many of the circulating cytokines would have degraded during incubation and would not be present at biological relevant levels at the time of assay.

Effect on Th1–Th2 response. Because cytokines are pleiotropic, we created functional Th1 and Th2 categories according to standard designations. Generally, Th1-type cytokine groups include proinflammatory cytokines such as IFN- γ and IL-2 [48]. Likewise, Th2-type cytokine groups generally include anti-inflammatory or regulatory cytokines, such as IL-4, IL-5 and IL-13 [48]. Our categories were based on the most conservative designations; our Th1-suite included IFN- γ and IL-2, while the Th2-suite included IL-4, IL-5 and IL-13 [48]. We ran parallel analyses with broader categories (Supplementary Table S10). This included a proinflammatory suite (IFN γ , IL-2, IL-6, IL-8, IL-

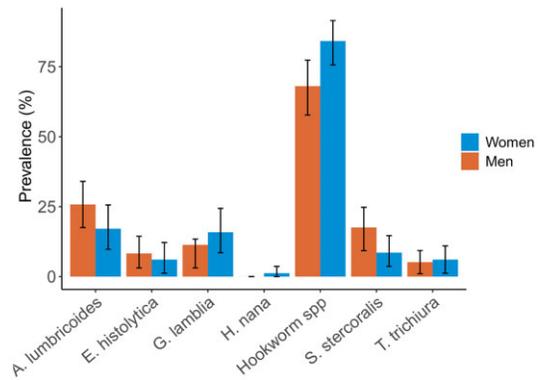


Figure 1. Prevalence of parasites identified microscopically in 179 fresh fecal samples

12p70, TNF α) and an anti-inflammatory suite (IL-4, IL-5, IL-10, IL-13).

We added a constant (+1) to and log-transformed each cytokine value. We calculated z-scores for each of the functional suites within each treatment with the sum of the individual cytokines. Finally, we averaged z-scores within each category to produce z-scored suite responses for each sample. Assessing women and men separately, we first modeled z-scores within each treatment by each STH (presence/absence), BMI, age and pregnancy (for women). We then performed similar analyses modeling the effect of eosinophil count on Th1/Th2-suite z-scores, including the same covariates as predictors and total leukocyte count. All analyses were done in R.

RESULTS

Descriptives

Helminth richness ranged from 0 to 4 species per person (median: 1, Fig. 1, $n=179$), and 87% of samples had at least one helminth infection. We identified hookworm (*Necator americanus* or *Ancylostoma duodenale*) (75.4% prevalence), *Ascaris lumbricoides* (21.8%), *Strongyloides stercoralis* (13.4%), *Trichuris trichiura* (5.6%) and *Hymenopolis nana* (0.6%). The protozoan *Giardia lamblia* (13.4%) and the amoeba *Entamoeba histolytica* (7.3%) were also identified. Coinfections (37%) were most likely to occur with *A. lumbricoides* and *T. trichiura* (Pearson correlation = 0.23, $P < 0.01$, Supplementary Table S1) and least likely to occur between *A. lumbricoides* and hookworms (Pearson correlation = -0.27 , $P < 0.01$, Supplementary Table S1). Only helminths with a prevalence of $>10\%$ were included for downstream analyses (*A. lumbricoides*, *S. stercoralis* and hookworm species; Supplementary Table S2).

Eosinophil counts ranged from 492 to 7200 cells/ μl , with a median of 1950 cells/ μl and a mean of 2230 cells/ μl . For comparison, the US reference range for eosinophils is <500 cells/ μl ; >500 cells/ μl is categorized as eosinophilia [49]. Thus, by US

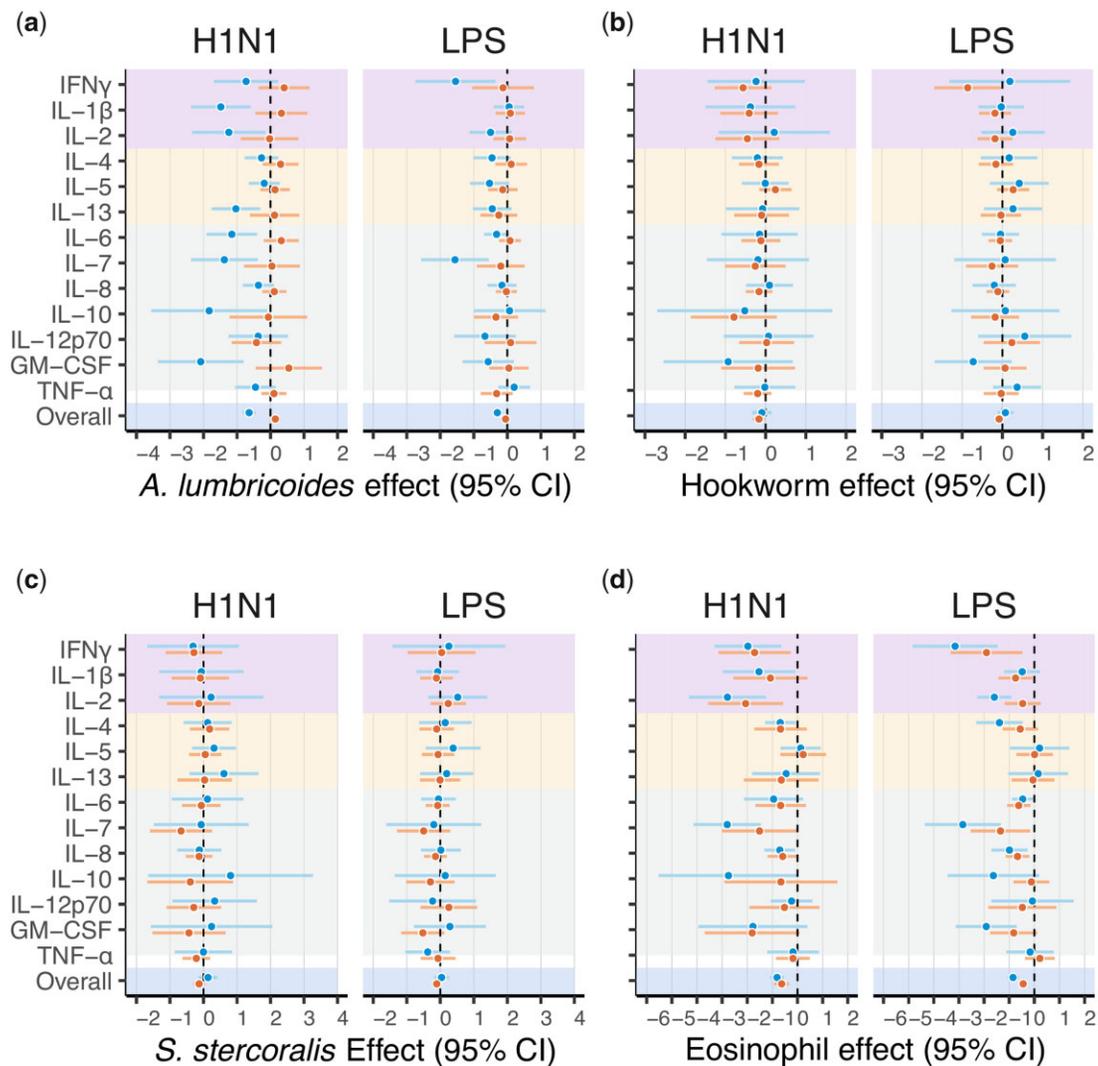


Figure 2. Individual cytokine responses to H1N1 and LPS stimulation as a function of (a) *A. lumbricoides*, (b) hookworms, (c) *S. stercoralis* and (d) eosinophil count. Coefficients and 95% confidence intervals are shown for each condition for women (blue) and men (orange). Cytokines are shaded by their Th1- (purple) or Th2-type (yellow) classification, and the overall effect size for each stimulation is highlighted in blue

standards all but two (98.6%) of the sampled Tsimane were eosinophilic.

Parasites and eosinophils

The presence of *S. stercoralis* was the only parasite infection positively associated with increased eosinophils ($P=0.004$) for men. No parasite infection was associated with changes in eosinophil count for women.

Individual cytokine responses

H1N1 stimulation. For women, the presence of *A. lumbricoides* was significantly ($P \leq 0.05$) negatively associated with the

response of seven cytokines to H1N1 (IL-1 β , IL-2, IL-6, IL-7, IL-10, IL-13 and GM-CSF; [Supplementary Table S4](#)). *Ascaris lumbricoides* was the sole parasite to exhibit a significant relationship with cytokine responses for women, and exhibited negative betas associated with all 13 cytokines ([Fig. 2](#) and [Supplementary Table S4](#)). The overall estimate of the impact of *A. lumbricoides* across cytokines for women in the fixed-effects model was -0.6 ($P < 0.01$; [Fig. 2](#) and [Supplementary Table S4](#)). Eosinophils were significantly negatively associated with the response of six cytokines to H1N1 (IFN- γ , IL-1 β , IL-2, IL-4, IL-7, IL-8), with eosinophils exhibiting negative betas for all of the 13 total cytokines but IL-5 ([Fig. 2](#) and [Supplementary Table S5](#)). The overall estimate of eosinophil impact across cytokines for women in the fixed-effects model was -0.8 ($P < 0.01$; [Fig. 2](#) and [Supplementary Table S4](#)). Age was positively associated with

TNF-alpha and GM-CSF responses to H1N1 in parasite-specific models (Supplementary Table S5).

For men, individual parasite infections were not significantly associated with cytokine responses to H1N1 stimulation (Supplementary Table S6). Eosinophils were significantly negatively associated with the response of two cytokines to H1N1 (IFN- γ and IL-2), and exhibited negative betas for all cytokines but IL-5 (Fig. 2 and Supplementary Table S7). The overall estimate of eosinophil impact across cytokines in the fixed-effects model was -0.63 ($P < 0.01$; Fig. 2 and Supplementary Table S7). Age, BMI, pregnancy, and leukocyte count had varying effects on cytokine response to H1N1 in all models (Fig. 2 and Supplementary Table S7).

LPS stimulation. For women, the presence of *A. lumbricoides* was significantly negatively associated with the response of two cytokines to LPS stimulation (IFN- γ and IL-7; Supplementary Table S4). No other parasite infection demonstrated any relationship with cytokine expression. The overall estimate of the impact of *A. lumbricoides* on cytokine response in the fixed-effects model was -0.3 ($P < 0.01$; Supplementary Table S4). Eosinophils were significantly negatively associated with the response of seven cytokines to LPS (IFN- γ , IL-4, IL-6, IL-7, IL-8 and GM-CSF), and produced negative betas for all cytokines except IL-5 and IL-13 (Fig. 2 and Supplementary Table S5). The overall estimate of eosinophil impact across cytokines in the fixed-effects model was -0.9 ($P < 0.01$; Supplementary Table S5). Age, BMI, pregnancy, and leukocyte count had varying effects on cytokine responses to LPS. Age was positively associated with TNF-alpha and GM-CSF responses to LPS in parasite-specific models (Supplementary Table S5). Additional models excluding pregnant women were not substantially different from the primary models.

For men, the presence of hookworms was significantly negatively associated with the expression of IFN- γ to LPS stimulation (Supplementary Table S6). No other parasite infection exhibited any relationship with cytokine expression. Eosinophils were significantly negatively associated with the response of five cytokines to LPS (IFN- γ , IL-1 β , IL-6, IL-7 and IL-8), and produced negative betas for all cytokines except IL-5 and TNF- α (Fig. 2 and Supplementary Table S7). The overall estimate of eosinophil impact across cytokines in the fixed-effects model was -0.44 ($P < 0.01$) for men (Fig. 2), and age, BMI, and leukocyte count had varying effects on cytokine response to LPS. Specifically, age was associated with higher IL-7 response to LPS stimulation (Supplementary Table S7).

Th1–Th2 responses

For women, *A. lumbricoides* was not associated with the Th1- or Th2-type cytokine suite to H1N1 stimulation, and was

negatively associated with the response of the Th1- type cytokine suite ($\beta = 0.6$, $P = 0.04$) to LPS stimulation but not with the response of the Th2-type cytokine suite (Supplementary Table S8). Eosinophil counts were negatively associated with the response of Th1-type ($\beta = -1.06$, $P < 0.01$) but not Th2-type ($\beta = -0.43$, $P = 0.13$) cytokines to H1N1, with the same pattern observed in response to LPS stimulation (Th1-type: $\beta = -1.45$, $P < 0.01$, Th2-type: $\beta = -0.41$, $P = 0.47$, Fig. 3 and Table S9).

For men, no parasites were significantly associated with the response of either cytokine suite to stimulation with either H1N1 or LPS. Eosinophil counts were negatively associated with response of Th1-type ($\beta = -1.13$, $P = 0.01$) but not Th2-type cytokines ($\beta = -0.3$, $P = 0.54$; Fig. 3 and Supplementary Table S8) to H1N1 stimulation. Similarly, eosinophil counts were associated with the response of Th1-type ($\beta = -0.64$, $P = 0.05$) but not Th2-type cytokines ($\beta = -0.22$, $P = 0.51$; Fig. 3 and Supplementary Table S9) to LPS stimulation. The other predictors had varying effects on the expression of Th1- and Th2-type cytokines for women and men. Higher age was associated with higher Th1 responses to H1N1 for women (a similar effect was observed for LPS but it did not meet the significance threshold); no association was observed in men in either medium (Supplementary Table S9).

DISCUSSION

As suggested by evolutionary hypotheses (e.g. ‘old friends’ and hygiene hypothesis), STH infections (specifically, *A. lumbricoides*) are associated with dampened proinflammatory responses to acute viral and bacterial stimulations among Tsimane women. Eosinophils, considered here as a secondary indicator of infection, were also significantly associated with lower cytokine responses to H1N1 and LPS in women and men. Together, these results suggest that helminth infections may attenuate the proinflammatory response to viruses and bacteria with the strongest effect in women.

Helminths may dampen the acute immune response to infection

STH infection in the Tsimane was high, with at least one helminth found in 87% and two or more helminths found in 37% of individuals. In conjunction with the high levels of immunoglobulin E (IgE) observed in the Tsimane [26, 28], high eosinophil levels suggest that STH prevalence is likely even higher than suggested by microscopy. The most common infections were with the gastrointestinal nematodes *A. lumbricoides*, *S. stercoralis*, *T. trichiura* and hookworm spp, which have all co-evolved with humans to elicit anti-inflammatory and regulatory immune responses characterized primarily by Th2 cytokine

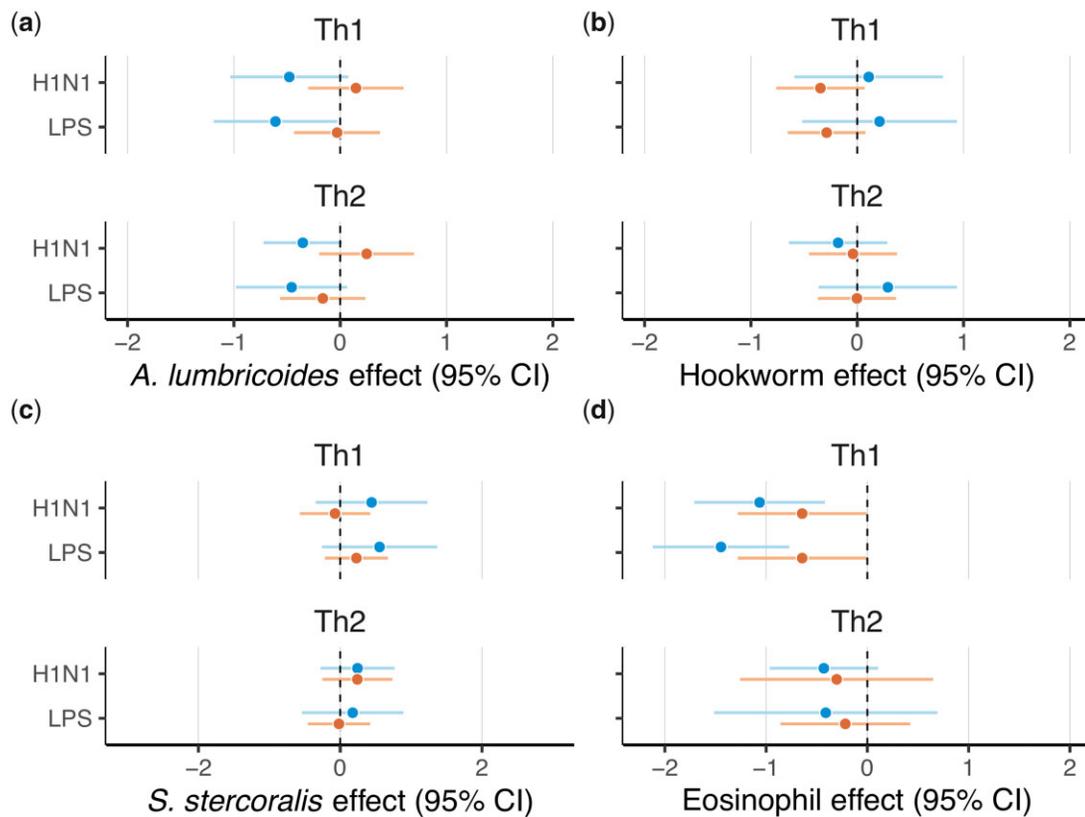


Figure 3. The effect of (a) *A. lumbricoides*, (b) Hookworm spp., (c) *S. stercoralis* and (d) eosinophils on Th1- and Th2-type responses to LPS and H1N1 stimulation. Coefficients and 95% confidence intervals (CIs) are shown for each condition for women (blue) and men (orange)

ascades [50–52] with some evidence for a role of Th1 cytokine responses [51]. These primarily anti-inflammatory immune responses can inhibit the ability of the immune system to launch proinflammatory responses in the face of some viral and bacterial pathogens.

The proinflammatory immune responses elicited by H1N1 and similar viruses are characterized by elevations in proinflammatory cytokines [53]. While a certain degree of responsiveness is vital to combating infection, a proinflammatory ‘cytokine storm’ can culminate in tissue and organ damage; indeed, deaths attributed to viral infections and certain gram-negative bacteria (e.g. *E. coli* [19, 54]) are typically associated with cytokine storms [17, 18]. In Tsimane women, infection with *A. lumbricoides* was significantly associated with impaired responses of certain cytokines implicated in cytokine storms [53, 55]. This suggests that Tsimane women infected with *A. lumbricoides* may be less susceptible to the development of cytokine storms during viral or bacterial infections. In Tsimane men, infection with hookworms was only associated with a diminished response of IFN- γ in LPS. In the Th1/Th2-suite analyses, the only observed effect was the dampening of the Th1-suite response to LPS in women by *A. lumbricoides*. The sex-specific effect of *A. lumbricoides* occurs despite fairly equal infection prevalence among women and men (Table 1), and may be tied to parasite-

specific sex differences in immunity [56, 57] and the profiles of chronic versus acute infections (Fig. 3).

As expected based on both the limitations of traditional microscopy and the binary nature of our parasite infection data, eosinophils were negatively associated with more cytokines than were individual parasite species. In women, higher eosinophil count was associated with decreased expression of both pro- and anti-inflammatory cytokines. In men, the effect was similarly spread across anti- and proinflammatory cytokines. In the functional Th1/Th2-suite analyses, eosinophils were associated with inhibited expression of the Th1-suite for both women and men in all media.

Associations between eosinophils and cytokine expression diverged from those between parasite presence and cytokine expression (i.e. certain cytokines were associated with eosinophils, but not with a specific parasite, and vice versa). This may be due to the limitations of our parasite detection techniques, or may be tied to factors that were not explicitly quantified here but may be reflected in our eosinophil measure. As infections with *A. lumbricoides*, *S. stercoralis*, and hookworms all involve an initial larval migration, our eosinophil analyses may capture infections at various points along the infection timeline, including prior to the reproductive phase characterized by high egg counts that would be captured with microscopy. Eosinophils

may also protect against new infections, represent the tail-end of recently cleared infections, or reflect early life exposure to helminths that would prime individuals for anti-inflammatory responses throughout their lives. These components of parasitism may underlie the relationship observed between antihelminthic treatment and eosinophil count in certain populations with high STH infection rates [39, 40]. Indeed, Tsimane have significantly elevated IgE by the age of 5, and cross-sectional studies suggest that IgE remains high across the life course [2] consistent with lifetime STH exposure and infection. These results suggest that eosinophils may reflect a to-be-determined component of STH infection and recapitulate the immunosuppressive associations in the STH analyses.

We found the strongest immunosuppressive associations in women, pointing to sex differences in immune function deviating from the expected pattern of immune hyperactivity in women [7]. Women are generally less susceptible to pathogens [58, 59] and mammalian females tend to host parasite infections at lower rates and with lower loads than males [60] (but see [61]), which have been linked to the proinflammatory properties of estradiol [62] and the immunosuppressive components and energetic costs of testosterone [42, 63]. However, women have historically incurred higher morbidity and mortality associated with viral pandemics (reviewed in [62, 42, 63]). Many of the data that have pointed to higher morbidity and mortality in women during viral pandemics, however, are from industrialized populations, which are typically characterized by low STH rates [3], as well as high autoimmune risk and low fertility [7]. Our results suggest that concurrent STH infections may mitigate the risk of overreaction to viral and bacterial infection and may buffer women from the otherwise higher likelihood of development of cytokine storms through suppression of cytokine responses.

Advantages and limitations

Studies that include data from non-industrialized populations are relatively rare and provide knowledge impossible to produce in a laboratory, but carry certain limitations (e.g. prior H1N1 exposure is unlikely but possible, low sample size of pregnant women precluded trimester-level analysis, limited data on early-life exposure, no causality assessed). Most studies on immune responses, allergies, and autoimmune disorders take place in populations that are wealthy and industrialized: in other words, under conditions that diverge sharply from those under which humans evolved [64]. Broadening studies of health and disease to populations that do not fit the wealthy and industrialized mold contributes to a more robust understanding of human variation and of effects on health and disease that originate in mismatch driven by rapidly changing human landscapes.

CONCLUSIONS

Altogether, our results support the hypothesis that STHs inhibit the cytokine response to viruses and bacteria by dampening the proinflammatory cytokine phenotype (consistent with lower vaccine immunogenicity associated with helminth infections [65]) and suggest that helminths may buffer the stronger immune responses of Tsimane women against runaway proinflammatory responses [32, 60]. These results add to a growing level of support for the 'old friends' and hygiene hypotheses, suggesting that STH infections may play an essential role in regulating immune function, and provide further evidence that the lack of STH infections in industrialized populations may increase the risk of over-reactive immune responses in the absence of STH immune priming [3–5]. Although we did not directly test this relationship, our results suggest that the helminth-induced anti-inflammatory immunomodulatory network may attenuate some of the most severe symptoms of viral infections such as SARS-CoV-2 [4, 8]. Variation in helminth prevalence may thus play a role in the complex network of community-specific factors contributing to global COVID-19 mortality patterns.

SUPPLEMENTARY DATA

Supplementary data is available at *EMPH* online.

ACKNOWLEDGEMENTS

We thank the Tsimane participants and communities who engaged in this research and the Tsimane Health and Life History Project staff and personnel for their efforts and logistical support. We thank Thais Mendes for laboratory assistance, Maria Yazdanbakhsh, Erliyani Sartono and Linda May for assistance with stimulation protocols, and Kenny Chiou for visualization assistance.

FUNDING

This research was funded by the NIH/NIA (R01AG024119, RF1AG054442) and the ASU Center for Evolution and Medicine. J.S. acknowledges IAST funding from the French National Research Agency (ANR) ANR-17-EURE-0010 (Investissements d'Avenir program).

CONFLICT OF INTEREST

None declared.

REFERENCES

1. Rook GAW, Lowry CA, Raison CL. Microbial "Old Friends", immunoregulation and stress resilience. *Evol Med Public Health* 2013;**2013**: 46–64.
2. Blackwell AD, Martin M, Kaplan H, Gurven M. Antagonism between two intestinal parasites in humans: the importance of co-infection for infection risk and recovery dynamics. *Proc Biol Sci* 2013;**280**:20131671.

3. Trumble BC, Finch CE. The exposome in human evolution: from dust to diesel. *Q Rev Biol* 2019;**94**:333–94.
4. Cepon-Robins TJ, Gildner TE. Old friends meet a new foe: a potential role for immune-priming parasites in mitigating COVID-19 morbidity and mortality. *Evol Med Public Health* 2020;**2020**:234–48.
5. Yazdanbakhsh M, Kreamsner PG, van Ree R. Allergy, parasites, and the hygiene hypothesis. *Science* 2002;**296**:490–4.
6. Garcia AR, Trumble B, Kraft TS *et al.* Does exposure to parasites modify relationships between diurnal cortisol and leukocytes among Honduran women? *Am J Phys Anthropol* 2020;**173**:463–79.
7. Natri H, Garcia AR, Buetow KH *et al.* The pregnancy pickle: evolved immune compensation due to pregnancy underlies sex differences in human diseases. *Trends Genet* 2019;**35**:478–88.
8. Bradbury RS, Piedrafito D, Greenhill A, Mahanty S. Will helminth co-infection modulate COVID-19 severity in endemic regions? *Nat Rev Immunol* 2020;**20**:342.
9. Maizels RM, McSorley HJ. Regulation of the host immune system by helminth parasites. *J Allergy Clin Immunol* 2016;**138**:666–75.
10. Berger A. Th1 and Th2 responses: what are they? *BMJ* 2000;**321**:424.
11. Mosmann TR, Sad S. The expanding universe of T-cell subsets: Th1, Th2 and more. *Immunol Today* 1996;**17**:138–46.
12. Cortés A, Muñoz-Antoli C, Esteban JG, Toledo R. Th2 and Th1 responses: clear and hidden sides of immunity against intestinal helminths. *Trends Parasitol* 2017;**33**:678–93.
13. White MPJ, McManus CM, Maizels RM. Regulatory T-cells in helminth infection: induction, function and therapeutic potential. *Immunology* 2020;**160**:248–60.
14. Zaccone P, Fehervari Z, Phillips JM *et al.* Parasitic worms and inflammatory diseases. *Parasite Immunol* 2006;**28**:515–23.
15. Strachan DP. Hay fever, hygiene, and household size. *BMJ* 1989;**299**:1259–60.
16. Wammes LJ, Hamid F, Wiria AE *et al.* Community deworming alleviates geohelminth-induced immune hyporesponsiveness. *Proc Natl Acad Sci USA* 2016;**113**:12526–31.
17. Channappanavar R, Perlman S. Pathogenic human coronavirus infections: causes and consequences of cytokine storm and immunopathology. *Semin Immunopathol* 2017;**39**:529–39.
18. Vaninov N. In the eye of the COVID-19 cytokine storm. *Nat Rev Immunol* 2020;**20**:277.
19. Eloiseily EM, Cron RQ. Bacteria-associated cytokine storm syndrome. In: RQ Cron, EM Behrens (eds.). *Cytokine Storm Syndrome*. Cham: Springer International Publishing, 2019, 307–17.
20. Hartgers FC, Yazdanbakhsh M. Co-infection of helminths and malaria: modulation of the immune responses to malaria. *Parasite Immunol* 2006;**28**:497–506.
21. Montresor A, Mupfasoni D, Mikhailov A *et al.* The global progress of soil-transmitted helminthiasis control in 2020 and World Health Organization targets for 2030. *PLoS Negl Trop Dis* 2020;**14**:e0008505.
22. Findlater A, Bogoch II. Human mobility and the global spread of infectious diseases: a focus on air travel. *Trends Parasitol* 2018;**34**:772–83.
23. Gurven M, Stieglitz J, Trumble B *et al.* The Tsimane health and life history project: integrating anthropology and biomedicine. *Evol Anthropol* 2017;**26**:54–73.
24. Blackwell AD, Trumble BC, Maldonado Suarez I *et al.* Immune function in Amazonian horticulturalists. *Ann Hum Biol* 2016;**43**:382–96.
25. Kraft TS, Stieglitz J, Trumble BC *et al.* Nutrition transition in 2 lowland Bolivian subsistence populations. *Am J Clin Nutr* 2018;**108**:1183–95.
26. Blackwell AD, Gurven MD, Sugiyama LS *et al.* Evidence for a peak shift in a humoral response to helminths: age profiles of IgE in the Shuar of Ecuador, the Tsimane of Bolivia, and the U.S. NHANES. *PLoS Negl Trop Dis* 2011;**5**:e1218.
27. Blackwell AD, Tamayo MA, Beheim B *et al.* Helminth infection, fecundity, and age of first pregnancy in women. *Science* 2015;**350**:970–2.
28. Blackwell AD, Trumble BC, Maldonado Suarez I *et al.* Immune function in Amazonian horticulturalists. *Ann Hum Biol* 2016;**43**:382–6.
29. Dinkel KA, Costa ME, Kraft TS *et al.* Relationship of sanitation, water boiling, and mosquito nets to health biomarkers in a rural subsistence population. *Am J Hum Biol* 2020;**32**:e23356.
30. Matsuura M. Structural modifications of bacterial lipopolysaccharide that facilitate gram-negative bacteria evasion of host innate immunity. *Front Immunol* 2013;**4**:109.
31. Nunn CL, Lindenfors P, Pursall ER, Rolff J. On sexual dimorphism in immune function. *Philos Trans R Soc Lond B Biol Sci* 2009;**364**:61–9.
32. Klein SL, Flanagan KL. Sex differences in immune responses. *Nat Rev Immunol* 2016;**16**:626–38.
33. Zhang Y-Y, Luo J-P, Liu Y-M *et al.* Evaluation of Kato–Katz examination method in three areas with low-level endemicity of schistosomiasis japonica in China: a Bayesian modeling approach. *Acta Trop* 2009;**112**:16–22.
34. Krauth SJ, Coulibaly JT, Knopp S *et al.* An in-depth analysis of a piece of shit: distribution of *Schistosoma mansoni* and hookworm eggs in human stool. *PLoS Negl Trop Dis* 2012;**6**:e1969.
35. Sinniah B. Daily egg production of *Ascaris lumbricoides*: the distribution of eggs in the faeces and the variability of egg counts. *Parasitology* 1982;**84**:167–75.
36. Klion AD, Nutman TB. The role of eosinophils in host defense against helminth parasites. *J Allergy Clin Immunol* 2004;**113**:30–7.
37. Schulte C, Krebs B, Jelinek T *et al.* Diagnostic significance of blood eosinophilia in returning travelers. *Clin Infect Dis* 2002;**34**:407–11.
38. Pardo J, Carranza C, Muro A *et al.* Helminth-related Eosinophilia in African immigrants, Gran Canaria. *Emerg Infect Dis* 2006;**12**:1587–9.
39. Tahapary DL, de Ruiter K, Martin I *et al.* Effect of anthelmintic treatment on insulin resistance: a cluster-randomized, placebo-controlled trial in Indonesia. *Clin Infect Dis* 2017;**65**:764–71.
40. Fincham JE, Markus MB, Adams VJ *et al.* Association of deworming with reduced eosinophilia: implications for HIV/AIDS and co-endemic diseases: research letters. *S Afr J Sci* 2003;**99**:182–4.
41. May L, van Bodegom D, Kuningas M *et al.* Performance of the whole-blood stimulation assay for assessing innate immune activation under field conditions. *Cytokine* 2009;**45**:184–9.
42. Trumble BC, Blackwell AD, Stieglitz J *et al.* Associations between male testosterone and immune function in a pathogenically stressed forager-horticultural population. *Am J Phys Anthropol* 2016;**161**:494–505.
43. Stieglitz J, Trumble BC, Thompson ME *et al.* Depression as sickness behavior? A test of the host defense hypothesis in a high pathogen population. *Brain Behav Immun* 2015;**49**:130–9.
44. Yockey LJ, Iwasaki A. Interferons and proinflammatory cytokines in pregnancy and fetal development. *Immunity* 2018;**49**:397–412.
45. Hové C, Trumble BC, Anderson AS *et al.* Immune function during pregnancy varies between ecologically distinct populations. *Evol Med Public Health* 2020;**2020**:114–28.

46. Balduzzi S, Rücker G, Schwarzer G. How to perform a meta-analysis with R: a practical tutorial. *Evid Based Ment Health* 2019;**22**:153–60.
47. Zhou X, Fragala MS, McElhaney JE, Kuchel GA. Conceptual and methodological issues relevant to cytokine and inflammatory marker measurements in clinical research. *Curr Opin Clin Nutr Metab Care* 2010;**13**:541–7.
48. Mosmann TR, Coffman RL. TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. *Annu Rev Immunol* 1989;**7**:145–73.
49. van Assendelft OW. Reference values for the total and differential leukocyte count. *Blood Cells* 1985;**11**:77–96.
50. Bradley JE, Jackson JA. Immunity, immunoregulation and the ecology of trichuriasis and ascariasis. *Parasite Immunol* 2004;**26**:429–41.
51. Gaze S, McSorley HJ, Daveson J *et al.* Characterising the mucosal and systemic immune responses to experimental human hookworm infection. *PLoS Pathog* 2012;**8**:e1002520.
52. Iriemenam NC, Sanyaolu AO, Oyibo WA, Fagbenro-Beyioku AF. Strongyloides stercoralis and the immune response. *Parasitol Int* 2010;**59**:9–14.
53. Lee N, Wong CK, Chan PKS *et al.* Cytokine response patterns in severe pandemic 2009 H1N1 and seasonal influenza among hospitalized adults. *PLoS One* 2011;**6**:e26050.
54. D'Elia RV, Harrison K, Oyston PC *et al.* Targeting the “Cytokine Storm” for therapeutic benefit. *Clin Vaccine Immunol* 2013;**20**:319–27.
55. Yu X, Zhang X, Zhao B *et al.* Intensive cytokine induction in pandemic H1N1 influenza virus infection accompanied by robust production of IL-10 and IL-6. *PLoS One* 2011;**6**:e28680.
56. Bancroft AJ, Artis D, Donaldson DD, Sypek JP *et al.* Gastrointestinal nematode expulsion in IL-4 knockout mice is IL-13 dependent. *Eur J Immunol* 2000;**30**:2083–91.
57. Asemota OO, Nmorsi OPG, Isaac C *et al.* Chemokines responses to ascaris lumbricoides sole infection and co-infection with hookworm among Nigerians. *N Am J Med Sci* 2014;**6**:84–8.
58. Klein SL, Huber S. Sex differences in susceptibility to viral infection. In: SL Klein, C Roberts (eds.). *Sex Hormones and Immunity to Infection*. Berlin, Heidelberg: Springer Berlin Heidelberg, 2010, 93–122.
59. Roberts CW, Walker W, Alexander J. Sex-associated hormones and immunity to protozoan parasites. *Clin Microbiol Rev* 2001;**14**:476–88.
60. Nunn CL, Lindenfors P, Rhiannon Pursall E, Rolff J. On sexual dimorphism in immune function. *Philos Trans R Soc B Biol Sci* 2009;**364**:61–9.
61. Escobedo G.D, León-Nava, MA Morales-Montor, J. Sex differences in parasitic infections: beyond the dogma of female-biased resistance. In: SL Klein, C Roberts (eds.). *Sex Hormones and Immunity to Infection*. Berlin, Heidelberg: Springer Berlin Heidelberg, 2010, 187–204.
62. Klein SL, Hodgson A, Robinson DP. Mechanisms of sex disparities in influenza pathogenesis. *J Leukoc Biol* 2012;**92**:67–73.
63. Muehlenbein MP, Bribiescas RG. Testosterone-mediated immune functions and male life histories. *Am J Hum Biol* 2005;**17**:527–58.
64. Gurven M, Lieberman DE. WEIRD bodies: mismatch, medicine and missing diversity. *Evol Hum Behav* 2020;**41**:330–40.
65. Elias D, Britton S, Aseffa A *et al.* Poor immunogenicity of BCG in helminth infected population is associated with increased in vitro TGF-beta production. *Vaccine* 2008;**26**:3897–902.