Published in final edited form as:

Am J Phys Anthropol. 2013 June ; 151(2): 183–190. doi:10.1002/ajpa.22250.

Predictors of Delayed-Type Hypersensitivity to *Candida albicans* and Anti-Epstein-Barr Virus Antibody Among Children in Kilimanjaro, Tanzania

Katherine Wander^{1,*}, Bettina Shell-Duncan¹, Eleanor Brindle², and Kathleen O'Connor^{1,2}

¹Department of Anthropology, University of Washington, Seattle, WA 98195

²Center for Studies in Demography and Ecology, University of Washington, Seattle, WA 98195

Abstract

We evaluated sex, age, nutritional status, and infectious disease (ID) as predictors of two biomarkers of cell-mediated immunity (CMI), delayed-type hypersensitivity to *Candida albicans* (DTH-*Candida*), and anti-Epstein-Barr virus antibody (EBV Ab), among 200 children in Kilimanjaro, Tanzania. DTH-*Candida*, which decreases with compromised CMI, was positively associated with age (OR: 1.27; 95% CI: 1.02, 1.57) and triceps skinfold (TSF; OR: 1.16; 95% CI: 1.02, 1.26), and inversely associated with height-for-age Z score (HAZ; OR: 0.86; 95% CI: 0.68, 1.08) and diagnosed ID (OR: 0.48; 95% CI: 0.22, 1.08). There was significant interaction between TSF and ID: DTH-*Candida* exhibited a strong inverse association with ID among children with low TSF (OR: 0.16; 95% CI: 0.05, 0.50) and a strong positive association with TSF among children with ID (OR: 2.64; 95% CI: 1.29, 5.42). EBV Ab, which increases with compromised CMI, was inversely associated with male sex (β : -0.47; 95% CI: -0.70, -0.24) and TSF (β : -0.04; 95% CI: -0.08, 0.00), and positively associated with HAZ (β : 0.06; 95% CI: -0.03, 0.15). Among males, EBV Ab was positively associated with anemia. Among normal HAZ children, EBV Ab was inversely associated with TSF. There was no association between DTH-*Candida* and EBVAb. While DTH-*Candida* provides a direct measure of CMI, our results suggest that interpretation of EBV-Ab among Kilimanjaro children was complicated by its indirect relationship with CMI. Among our sample, CMI increased with age and adequate nutrition and was compromised during acute ID. The suggestive CMI-compromising effect of increasing height-for-age may bear further exploration.

Keywords

immune function; cell-mediated immunity; infectious disease; malnutrition

One of the most important factors in susceptibility to infectious disease (ID) is the immune system's ability to mount protective responses against infectious agents. Immune responses rely on many components. Innate immunity physically isolates (via inflammation) and

damages or destroys (via complement cascade and phagocytes) infectious agents; innate immunity also presents (via dendritic cells) antigens to the adaptive immune system (Janeway et al., 2001). Adaptive immunity is responsible for recognizing and “remembering” novel pathogen antigens. Humoral immunity targets pathogens directly (via antibody, produced by B-cells); cell-mediated immunity (CMI) recognizes and destroys host cells displaying evidence of infection with a pathogen (via cytotoxic T-cells) (*ibid.*). CMI is the component of the immune response that is most readily perturbed; compromised CMI increases risk of ID (Fine et al., 1994; Shell-Duncan and Wood, 1997). For these reasons, CMI is often the focus of research into immune function and ID susceptibility.

There exists no single widely accepted “gold standard” measure of CMI. Profound immunodeficiency, such as that associated with advanced HIV disease, can be identified by low numbers of immune cells (white blood cells; leukopenia); however, the absence of leukopenia does not alone indicate CMI is intact. Measures of immune activity, such as white blood cell count or acute phase reactants (e.g., C-reactive protein), can be used to identify on-going responses to infectious stimuli (i.e., subjects likely undergoing ID). However, these measures are of little use in investigation of the *capacity* to mount a cell-mediated immune response.

Recent anthropological research has investigated variability in immune function, as well as predictors and outcomes associated with this variability (Shell-Duncan, 1993, 1995, 1997; Shell-Duncan and Wood, 1997; McDade, 2001, 2002; McDade et al., 2000a, 2001a, 2008; Gurven et al., 2008; Muehlenbein et al., 2010; Wander et al., 2012a). Such research is inherently population-based, and is often conducted in remote and challenging field settings (where infrastructure is poor, and access to healthcare is limited). Children are often the subjects of such research (e.g., Shell-Duncan, 1993; 1995; 1997; Shell-Duncan and Wood, 1997; Wander et al., 2012a), as they are particularly vulnerable to ID, often due to immature or compromised CMI. The practical constraints of population-based research, remote settings, and young research subjects complicate the use of biomarkers of CMI in anthropological research. We evaluated predictors of two biomarkers of CMI, delayed-type hypersensitivity to *Candida albicans* (DTH-*Candida*) and anti-Epstein-Barr virus antibody (EBV Ab) among a population-based sample of children in rural Kilimanjaro, Tanzania.

Delayed-type hypersensitivity (DTH) reactions can be evaluated with a simple skin test. A small amount of pathogen antigen is introduced under the skin; if a test subject has previously responded to the antigen, and CMI is intact, local activation of a secondary (or memory) immune response by CMI will be evident as an induration at the test site after 24–72 h (Christou et al., 1985). DTH tests are useful in two scenarios: if intact CMI can be assumed, DTH is useful in evaluating exposure to a pathogen; if exposure to a pathogen can be assumed, DTH is useful in evaluating CMI. For example, in the US, most adults are assumed to have competent cell-mediated immune responses, and DTH to tuberculin purified protein derivative (PPD) is used to screen for *Mycobacterium tuberculosis* exposure (Lee and Holzman, 2002). Conversely, DTH testing with an antigen to which exposure can be assumed, such as the ubiquitous fungal pathogen, *Candida albicans*, is used to evaluate CMI (e.g., Edelman et al., 1973; Segerstrom, 2008). *C. albicans* is the causative agent of “thrush” (oral candidiasis) and “yeast infection” (vaginal candidiasis); among severely

immunocompromised individuals, it can cause life-threatening systemic infection (candidemia). Occasionally (when neither intact CMI nor exposure can be assumed), DTH to tuberculin PPD and *C. albicans* are evaluated simultaneously; for example, to screen for *M. tuberculosis* exposure among HIV-infected individuals (e.g., Huebner et al., 1994).

Delayed-type hypersensitivity is an appealing biomarker of CMI because it allows direct observation of a subject's cell-mediated immune response to a pathogen antigen. The drawbacks of employing DTH in population-based studies of immune function are (1) interpretation: previous exposure to the recall antigen (e.g., *C. albicans*) must be assumed, but can rarely be proven; and (2) practicality: DTH testing can be expensive, often requires injection of the test antigen under the skin with a syringe (introducing variation and potential error, e.g., in the depth of intradermal administration), and poses a small risk of anaphylaxis to test subjects. These characteristics make DTH testing difficult to employ in population-based research, and are particularly problematic in remote field settings, and among young subjects.

The high prevalence of another infectious agent, Epstein-Barr virus (EBV), provides a second biomarker of CMI. By adulthood, ~90% of people have acquired chronic EBV infection; this infection is kept largely in check by the activity of cytotoxic T lymphocytes (Rickinson and Moss, 1997). When CMI is compromised, EBV replication increases, inducing increased production of anti-EBV immunoglobulin G antibody (EBV Ab). Thus, elevated EBV Ab is associated with an immunocompromised state (Glaser et al., 1991, 1993; McDade et al., 2000a), and has been used as a biomarker of CMI.

Like DTH, interpretation of EBV Ab is limited by the necessary assumption that subjects have experience with the pathogen: while low levels of EBV Ab indicate intact CMI, extremely low levels of EBV Ab cannot be interpreted, as they may indicate a subject is uninfected with EBV, and whether immunocompromised or not, is not producing EBV Ab. In addition, EBV Ab interpretation relies on an indirect mechanism—it is not the cell-mediated immune response *per se* that is observed, but a consequence of its failure—elevated antibody production—which is itself an aspect of immune function. EBV Ab is appealing as a biomarker of CMI for its practicality: it can be easily, accurately, and cheaply measured in whole blood stored as dried blood spots (DBS), lending it to use in population-based research in a wide range of field settings and among subjects of all ages.

Work in multiple settings has demonstrated consistent patterns: cell-mediated immune function increases with age during early childhood; and, male sex, undernutrition, and acute infection are risk factors for CMI failure (Washburn et al., 1965; Neumann et al., 1975; Kniker et al., 1985; Pinner et al., 1996; Zaman et al., 1997; Shell-Duncan, 1997). On the basis of this literature, we expected to observe among children in Kilimanjaro, Tanzania: (1) increased CMI among older children (manifest as a positive association between age and DTH-*Candida*, and an inverse association between age and EBV Ab); (2) lower measures of CMI among male children; (3) a positive association between CMI and body size (e.g., height-for-age), as well as adiposity; and, (4) lower measures of CMI among anemic children and those undergoing infectious disease; we also predicted, (5) an inverse association between DTH-*Candida* and EBV Ab.

MATERIALS AND METHODS

Participants

This project was carried out in the Machame area of Kilimanjaro, Tanzania. 314 2- to 7-year-old children participated in the project, randomly sampled from a census of all 2- to 7-year-old children in the study area. Children were eligible to participate if they were living with at least 1 parent and had been living in the study area for at least 6 months.

Data collection

Data were collected over the course of 4 weeks in spring of 2010. Children and their primary caregiving parents participated in 2 days of data collection. Data were collected by the lead author and 4 field assistants (residents of the study area and medical personnel trained in data collection techniques) at a healthcare facility belonging to Nshara Community Medical Center (NCMC). Written informed consent was obtained from parents of all participating children. Procedures and data collection protocols were approved by the Institutional Review Board of the University of Washington and the Tanzanian National Institute for Medical Research (NIMR) and research permission obtained from the Tanzanian Commission for Science and Technology (COSTECH).

Caregivers provided household and family demographic information and described the child's recent health and medical history. Each child's finger was pricked with a sterile lancet to obtain capillary whole blood; blood was immediately tested for HIV (SD BioLine HIV-1/2 3.0 rapid HIV-1/2 test) and hemoglobin concentration (HemoCue Hb 201⁺ hemoglobin system). Additional whole blood was allowed to fall freely onto filter paper (Whatman #903 Protein Saver Cards) and stored as dried blood spots (DBS). DBS were allowed to dry at room temperature for <24 h, and were then stored frozen until transported by courier on dry ice to the University of Washington for laboratory analysis. The Candin (Allermed Laboratories, San Diego, CA) skin test for DTH to *C. albicans* antigen was administered intra-dermally on each child's forearm by injecting 0.1 ml under the most superficial layers of the skin with a 27 gauge tuberculin syringe. On the second data collection visit, ~24 h after the first, the site of the Candin skin test was evaluated for the presence of an induration; the size of any induration measured in millimeters (mm) across 2 perpendicular diameters. Weight was measured with a digital scale and height measured with an anthropometer. Triceps skinfold (TSF) thickness was measured using a Lange Skinfold Measurement Caliper (Graham-Field). All anthropometric measurements and skin test evaluations were performed by the lead author to maintain measurement consistency. Children experiencing symptoms of ID, HIV positive children, and anemic children were referred to NCMC for additional care. With parents' permission, diagnoses for referred children were recorded.

Laboratory analysis

DBS specimens were analyzed in the Biological Anthropology and Biodemography Laboratory at the University of Washington for EBV Ab using a kit modified for use with DBS (DiaSorin ETI-VCA-G Catalog No. P001606A; McDade et al., 2000b). Specimens and controls were eluted in buffer, and eluent, calibrator, and kit controls were applied to

microtiter plates coated with Epstein-Barr viral capsid antigen (VCA p18). Horseradishperoxidase (HRP)-conjugated signal antibody (affinity-purified goat anti-human IgG) and substrate solution were added to develop a color signal proportional to EBV Ab concentration.

Dried blood spot specimens were also analyzed for C-reactive protein (CRP) and α_1 -acid glycoprotein (AGP, or orosomucoid), two biomarkers of inflammation used to identify acute infection. CRP was assessed with an in-house DBS assay (Brindle et al., 2010). Specimens and controls were eluted in buffer, and eluent, calibrator (Fitzgerald, cat. no. 30-AC10), and controls were applied to microtiter plates coated with mouse anti-human CRP monoclonal antibody (Bioscience Clone C5, cat. no. M86005M), followed by signal antibody (Bioscience mouse anti-human CRP monoclonal antibody; Clone C6, cat. no. M86284B). HRP-conjugated streptavidin (Invitrogen Corporation, cat. no. 43-8323) and substrate solution were added to develop a color signal proportional to CRP concentration. AGP was assessed with a commercially available enzyme immunoassay kit, modified for use with DBS specimens. After eluting, specimens, calibrators (GenWay Biotech human orosomucoid antigen, cat. no. 10-288-22927F), and controls were applied to microtiter plates coated with chicken anti-human orosomucoid antibody (GenWay Biotech, cat. no. 15-288-22927F). HRP-conjugated chicken anti-human orosomucoid signal antibody (GenWay Biotech, cat. no. 27-288-22927F) and substrate solution were added to develop a color signal proportional to AGP concentration.

All specimens, calibrators, and controls were evaluated in duplicate. Concentrations were estimated from color signal optical density using a 4-parameter logistic model in Gen5 Software (BioTek, Winooski, VT). For all plates, an extrapolation factor of 1 was used to estimate concentrations of unknown specimens, with the exception of one plate of the EBV assay, containing ~15 specimens, for which an extrapolation factor of 2 was used, to estimate values of specimens that remained higher than standard curve after multiple dilutions. Intra-assay coefficients of variation (CV; for the assay runs included in this analysis) were 3.6% for EBV, 16.2% for CRP, and 10.6% for AGP. Interassay CVs were 12.0% at low and 6.7% at high concentrations of EBV Ab; 11.9% at low and 10.2% at high concentrations of CRP; 18.6% at low and 9.8% at high concentrations of AGP.

Data analysis

A mean induration ≥ 5 mm defined DTH-*Candida* positivity; induration size was also evaluated as a continuous variable. EBV Ab was considered negative, or undetectable, per the assay kit instructions based on optical density below that of the “positive” calibrator (Calibrator II of the standard curve); negative EBV Ab results were excluded from further analysis. For those specimens positive for EBV Ab, EBV Ab values were natural logarithm-transformed (due to substantial right skew in the distribution of EBV Ab). Natural log-transformed EBV Ab was evaluated as a continuous variable. Age was calculated from the child’s reported date of birth. Elevated CRP (> 1.1 mg l⁻¹) or AGP (> 0.76 g l⁻¹) values were used to identify acute infection (based on Wander et al., 2012b); ID diagnoses were identified among children referred to NCMC at the time of data collection as a second indicator of acute ID morbidity. TSF was analyzed as a continuous variable. Z scores were

calculated for weight-for-height (WHZ) and height-for-age (HAZ) and wasted (WHZ <-2) and stunted (HAZ <-2) children identified. Age-specific definitions of low hemoglobin were used to identify anemia (Nestel, 2002).

Data analysis was performed using Stata 11.2 software (Statacorp; College Station, TX). Linear regression was used to assess predictors of DTH-*Candida* induration size and EBV Ab. Logistic regression was used to assess predictors of positive DTH-*Candida*. Age and sex were included in all multivariate models, as were the nutritional (WHZ, HAZ, stunting, TSF, anemia) and ID (elevated biomarkers, diagnosis upon referral) variables which exhibited univariate associations with $P \leq 0.20$ with at least 1 outcome. Significance was defined as $P \leq 0.05$; $0.05 < P \leq 0.10$ was considered “marginal” significance. All potential interaction terms in each multivariate model were evaluated; because estimated coefficients or ORs for interaction terms in logistic regression are not readily interpretable, separate models were constructed to evaluate the impact of predictor variables involved in significant interactions.

RESULTS

Complete information for all predictor and outcome variables was available for 200 children. Characteristics of these children are shown in Table 1. Data from 1 HIV positive child were excluded. Additional exclusions included: refused TSF measurement (51 children), unknown date of birth (17 children), inadequate sample for measurement of all laboratory analytes (21 children), DTH test administration problems (9 children), and EBV negativity (26 children). Children excluded due to missing information were significantly younger (younger children may have been more likely to refuse TSF measurement) and were lower in HAZ, and thus, more likely to be stunted. Excluded children did not differ significantly from those with complete information in biomarkers of CMI or in any other predictors considered.

Univariate associations between predictors of interest and biomarkers of CMI are shown in Table 2. Positive DTH-*Candida* was significantly positively associated with TSF and inversely associated with ID (as diagnosed upon referral on the day of data collection; this association was only marginally significant). As a continuous variable, DTH-*Candida* induration size was significantly inversely associated with HAZ, and significantly positively associated with TSF. EBV Ab was significantly inversely associated with male sex.

Results of multivariate analysis for DTH-*Candida* are shown in Table 3. Both DTH-*Candida* outcomes (positivity and induration size, Table 3) were significantly positively associated with age and TSF. DTH-*Candida* induration size was significantly inversely associated with HAZ. DTH-*Candida* positivity was inversely associated with ID diagnosis; this association was of marginal significance.

When included in multivariate models of DTH-*Candida* positivity, the interaction term diagnosed ID*TSF was significant ($P = 0.012$). Separate models evaluating associations between positive DTH-*Candida* and ID for children with TSF above and below the median observed value (11.3 mm) are presented in Table 4a; models evaluating associations

between positive DTH-*Candida* and TSF for children with and without a diagnosed ID are presented in Table 4b. A substantial and significant inverse association between DTH-*Candida* positivity and ID was apparent only among children with TSF below the median value. A significant positive association between DTH-*Candida* positivity and TSF was apparent only among children with diagnosed ID. Results were similar for the DTH-*Candida* induration size outcome (data not shown). No significant interactions were apparent between other predictors of DTH-*Candida*.

Results of multivariate analysis for EBV Ab are shown in Table 5. Male sex was significantly inversely associated with EBV Ab (such that male sex was associated with 37% lower EBV Ab). TSF was inversely associated with EBV Ab; this association was of marginal significance.

When included in multivariate models of EBV Ab, the interaction term sex*anemia was significant ($P = 0.033$); the association between TSF and EBV Ab also differed substantially, although not significantly, by HAZ (stunting vs. not). Table 6 shows models evaluating associations between anemia and EBV Ab by sex; and also shows models evaluating associations between TSF and EBV Ab by stunting. A positive significant association between EBV Ab and anemia was apparent among male children. An inverse association of marginal significance between TSF and EBV Ab was apparent only among normal height-for-age children.

There was no significant association between EBV Ab and DTH (pairwise correlation between EBV Ab and DTH-*Candida* induration size: 0.0297, $P = 0.6761$; t test of EBV Ab by DTH-*Candida*: -0.3334 , $P = 0.7392$).

DISCUSSION

On the basis of work conducted in multiple populations, we expected cell-mediated immune function among Kilimanjaro children to increase with age, and to be diminished among male children, undernourished children, and those undergoing acute infection (Washburn et al., 1965; Neumann et al., 1975; Kniker et al., 1985; Pinner et al., 1996; Zaman et al., 1997; Shell-Duncan, 1997). Based on these predictions, the associations we expected to observe are summarized in Table 7.

Table 7 also summarizes our observations. Male sex was unassociated with DTH-*Candida* and inversely associated with EBV Ab. Age was positively associated with DTH-*Candida*, and unassociated with EBV Ab. TSF was positively associated with DTH-*Candida*, particularly among children undergoing ID, and not significantly associated with EBV Ab (an inverse association of marginal significance was observed). HAZ was inversely associated with DTH-*Candida* and positively associated with EBV Ab among male children. Anemia was unassociated with DTH-*Candida* and positively associated with EBV Ab among male children. ID diagnosis was inversely associated with DTH-*Candida* among children with low adiposity and unassociated with EBV Ab.

As expected, our analysis suggests that cell-mediated immune function increases with age among Kilimanjaro children, as evidenced by a positive association between age and DTH-

Candida. Male sex was not associated with higher risk of CMI failure among the children in our project, and, indeed, may have been protective: male sex was significantly and consistently inversely associated with EBV Ab. The absence of the expected association between male sex and CMI failure may be attributable to the young age of children sampled (excess ID mortality among males seems to begin in adolescence; Owens, 2002).

Our observations are largely consistent with the expected negative impact of inadequate nutrition on CMI: TSF (a measure of adiposity) was consistently positively associated with DTH-*Candida* and, among normal height-for-age children, inversely associated with EBV Ab. However, height exhibited an unexpected *inverse* association with CMI: HAZ was inversely associated with DTH-*Candida* and, among male children, positively associated with EBV Ab. The significance of these associations was notably inconsistent across analyses. The inverse association between height and CMI may reflect compromised immune function associated with growth in stature. Life history theory, which posits that limited resources are allocated among competing demands during childhood to optimize lifetime reproductive success, may interpret the inverse association between height and CMI as a result of the trade-off between growth and somatic maintenance (i.e., immune function). The limited statistical significance of associations between HAZ and biomarkers of CMI, however, lead us to interpret this result with caution until replicated with additional research.

Anemia, which may result from nutritional deficiency in iron or other micronutrients, or from blood loss and inflammation attributable to acute or chronic disease, exhibited inconsistent associations with biomarkers of CMI. Anemia was positively associated with DTH-*Candida*, suggesting a positive effect of anemia on CMI capacity. However, anemia was also positively associated with EBV Ab among males, suggesting a negative effect of anemia on CMI among male children. These associations were inconsistent and often of marginal significance, and, as such, must be interpreted with caution unless replicated with additional research.

As expected, acute ID morbidity was associated with compromised CMI: DTH-*Candida* was inversely associated with diagnosed ID. This effect was consistent, but of marginal significance in many models. There was significant and important interaction between the effects of ID and TSF on DTH-*Candida*: the inverse association between ID and DTH-*Candida* was apparent only among children with low TSF, while the positive association between TSF and DTH-*Candida* was apparent only among children with acute ID. This observation suggests that the combination of undernutrition and ID is particularly detrimental to CMI, increasing susceptibility to ID and compromising health among the subset of children who are both undernourished and undergoing acute infection (for additional discussion of the synergistic effects of malnutrition and infection in children's health, see Scrimshaw and SanGiovanni, 1997).

Our results support use of DTH-*Candida* as a biomarker of CMI among East African children. DTH testing with Candin proved affordable and practical, with relatively little loss of sample size due to skin test administration problems. We employed this test in a small rural health clinic, supported by trained healthcare providers; however, DTH skin testing has successfully been used outside of a healthcare setting, as well (Shell-Duncan, 1993, 1995,

1997). Associations between DTH-*Candida* and known predictors of CMI were largely consistent with our expectations: DTH-*Candida* was positively associated with age and adiposity, and inversely associated with clinically-apparent ID. It remains possible that DTH-*Candida* reflects variation in exposure to *C. albicans*, rather than in capacity to mount cell-mediated immune responses. However, our results are inconsistent with this interpretation; for example, there is no reason to expect exposure to *C. albicans* to be more common among children with higher adiposity, or less common among those with clinically apparent ID.

The interpretation of EBV Ab as a biomarker of CMI among children in Kilimanjaro has proved difficult. A substantial proportion of our sample (~8%) was excluded from analysis due to EBV negativity. EBV Ab increased with indicators of poor nutrition (lower TSF, anemia), consistent with compromised CMI among malnourished and anemic children. However, these associations were limited to a subset of children (normal height-for-age and male children, respectively). EBV Ab was inversely associated with male sex, suggesting a protective effect of male sex against compromised CMI, when the literature led us to predict the opposite or no effect of sex on CMI among our sample. Finally, we observed no association between EBV Ab and DTH-*Candida*, a direct indicator that did perform more strongly as expected for a biomarker of CMI. This may be due to the fact that EBV Ab does not reflect the same CMI processes as DTH-*Candida*.

Relationships between stressors such as undernutrition or acute infection, CMI, and EBV Ab production may be complicated in this population in several important ways. First, chronic undernutrition among children in our sample may have both indirect and direct effects on EBV Ab. EBV Ab is an indirect biomarker of CMI—as CMI is compromised, immune control of chronic EBV diminishes and viral load increases, stimulating increased production of EBV Ab. However, as malnutrition escalates, humoral immunity can be compromised, directly affecting antibody production, including EBV Ab. Thus, among more severely or chronically malnourished children, these direct (via humoral immunity) and indirect (via CMI) effects of malnutrition on EBV Ab production may “cancel out,” resulting in no net association between nutritional status and EBV Ab. This may explain our observation that the association between TSF and EBV Ab was apparent only among normal height for age (vs. stunted) children. EBV Ab may serve as a “better” biomarker of CMI among well-nourished than undernourished children.

Second, the age of children in our sample may complicate interpretation of EBV Ab. The utility of EBV Ab as a biomarker of CMI is contingent on the assumption of near-universal chronic EBV infection. The rate of seropositivity we observed (~92%) indicates a high prevalence of EBV in our study population. However, it is possible that a subset of the prevalent infections we captured may have been in the acute, rather than chronic, stage, exhibiting elevated EBV Ab production regardless of capacity for CMI. The lack of any association between EBV Ab and acute ID indicators (elevated biomarkers of inflammation or clinically apparent ID upon referral) argues against this conclusion, however.

Finally, it is possible that EBV Ab does not perform as predicted for a biomarker of CMI because it is affected by other physiological processes, obscuring any effect of CMI on EBV

Ab production. The majority of studies employing EBV Ab as a biomarker of CMI explore the effect of psychosocial stress on immunity to ID. Such stress may promote EBV proliferation directly (Kupfer and Summers, 1990; Schuster et al., 1991; Glaser et al., 1995), as well as through compromised CMI, potentially over-estimating the effects observed in such studies.

Neither DTH-*Candida* nor EBV Ab provides an ideal stand-alone biomarker of CMI function. Both are subject to limitations in interpretation, and neither performed entirely as expected in our analyses. Future work should focus on the development and interpretation of biomarkers that reflect multiple aspects of immune reactivity, to inform study of the immune system's overall capacity to protect against ID.

In summary, our evaluation of age, sex, nutritional, and ID predictors of two biomarkers of CMI, DTH-*Candida* and EBV Ab, among children in Kilimanjaro, Tanzania suggest the following:

- CMI increases with age and adiposity and diminishes with clinically apparent ID.
- Acute ID and malnutrition appear to act in synergy, with the combination of both clinically apparent ID and low adiposity significantly compromising CMI.
- CMI may diminish with increasing height for age; if replicated with additional research, this pattern may be evidence of a life-history trade-off between growth and immune function.
- DTH-*Candida* proved both practical and interpretable as a biomarker of CMI; the interpretation of EBV Ab was difficult in this setting, potentially complicated by the high prevalence of stunting and young age of children studied.

Acknowledgments

Grant sponsor: National Science Foundation Doctoral Dissertation Improvement Grant; Grant number: 0968742; Grant sponsor: Wenner-Gren Foundation for Anthropological Research Dissertation Fieldwork Grant; Grant number: 8065; Grant sponsor: Eunice Kennedy Shriver National Institute of Child Health and Human Development Research Infrastructure Grant; Grant number: 5R24 HD042828; Grant sponsor: Center for Studies in Demography and Ecology at the University of Washington.

The authors thank participating children and their families for their support and tremendous patience throughout data collection. We are indebted to the physicians and staff of Nshara Community Medical Center (in particular, Dr. Amini Uronu, Dr. Justo Kwayu, Dr. Judika Sindato, Zawia Massawe, and Rose Uronu), as well as Thadei Shirima, Evelin Uronu, Leonard Kweka, Mrs. Hamadi Ulomi, Zulf Uronu, and Steven Goodreau (UW Anthropology and Center for Studies in Demography and Ecology) for support and guidance during the conduct of this research, as well as the editors and anonymous reviewers for help in preparation of this manuscript.

LITERATURE CITED

- Christou NV, Boisvert G, Broadhead M, Meakins JL. Two techniques of measurement of the delayed hypersensitivity skin test response for the assessment of bacterial host resistance. *World J Surg.* 1985; 9:798–804. [PubMed: 4060750]
- Edelman R, Suskind RM, Olson RE, Sirisinha S. Mechanisms of defective delayed cutaneous hypersensitivity in children with protein-calorie malnutrition. *Lancet.* 1973; 301:506–509. [PubMed: 4119947]
- Fine PEM, Sterne JAC, Ponnighaus JM, Rees RJW. Delayed-type hypersensitivity, mycobacterial vaccines, and protective immunity. *Lancet.* 1994; 344:1245–1249. [PubMed: 7967984]

- Glaser R, Kutz LA, MacCallum RC, Malarkey WB. Hormonal modulation of Epstein-Barr virus replication. *Neuroendocrinology*. 1995; 62:356–361. [PubMed: 8544949]
- Glaser R, Pearson GR, Bonneau RH, Esterling BA, Atkinson C, Kiecolt-Glaser JK. Stress and the memory T-cell response to the Epstein-Barr virus in healthy medical students. *Health Psychol*. 1993; 12:435–442. [PubMed: 8293726]
- Glaser R, Pearson GR, Jones JF, Hillhouse J, Kennedy S, Mao H, Kiecolt-Glaser JK. Stress-related activation of Epstein-Barr virus. *Brain Behav Immun*. 1991; 5:219–232. [PubMed: 1654167]
- Gurven M, Kaplan H, Winking J, Finch C, Crimmins EM. Aging and inflammation in two epidemiological worlds. *J Gerontol A Biol Sci Med Sci*. 2008; 63:196–199. [PubMed: 18314457]
- Janeway, CA.; Travers, P.; Walport, M.; Shlomchik, M. *Immunobiology: the immune system in health and disease*. 5. New York: Garland Publishing; 2001.
- Kniker WT, Lesourd BM, McBryde JL, Corriel RN. Cell-mediated immunity assessed by Multitest CMI skin testing in infants and preschool children. *Am J Dis Child*. 1985; 139:840–845. [PubMed: 4025264]
- Kupfer SR, Summers WC. Identification of a glucocorticoid-responsive element in Epstein-Barr virus. *J Virol*. 1990; 64:1984–1990. [PubMed: 2157866]
- Lee E, Holzman RS. Evolution and current use of the tuberculin test. *Clin Infect Dis*. 2002; 34:365–370. [PubMed: 11774084]
- McDade TW. Lifestyle incongruity, social integration, and immune function in Samoan adolescents. *Social Sci Med*. 2001; 53:1351–1362.
- McDade TW. Status incongruity in Samoan youth: a bio-cultural analysis of culture change, stress, and immune function. *Med Anthropol Q*. 2002; 16:123–150. [PubMed: 12087626]
- McDade TW, Beck MA, Kuzawa C, Adair LS. Prenatal undernutrition, postnatal environments, and antibody response to vaccination in adolescence. *Am J Clin Nutr*. 2001; 74:543–548. [PubMed: 11566655]
- McDade TW, Reyes-García V, Tanner S, Huanca T, Leonard WR. Maintenance versus growth: investigating the costs of immune activation among children in lowland Bolivia. *Am J Phys Anthropol*. 2008; 136:478–484. [PubMed: 18383156]
- McDade TW, Stallings JF, Angold A, Costello EJ, Burleson M, Cacioppo JT, Glaser R, Worthman CM. Epstein-Barr virus antibodies in whole blood spots: a minimally invasive method for assessing an aspect of cell-mediated immunity. *Psychosomatic Med*. 2000b; 62:560–567.
- McDade TW, Stallings FJ, Worthman CM. Culture change and stress in Western Samoan youth: methodological issues in the cross-cultural study of stress and immune function. *Am J Hum Biol*. 2000a; 12:792–802. [PubMed: 11534070]
- Muehlenbein MP, Hirschtick JL, Bonner JZ, Swartz AM. Toward quantifying the usage costs of human immunity: altered metabolic rates and hormone levels during acute immune activation in men. *Am J Hum Biol*. 2010; 22:546–556. [PubMed: 20309883]
- Nestel, P. *Adjusting hemoglobin values in program surveys*. Washington, DC: International Nutritional Anemia Consultative Group; 2002.
- Neumann CG, Lawlor GJ Jr, Stiehm ER, Swenseid ME, Newton C, Herbert J, Ammann AJ, Jacob M. Immunologic responses in malnourished children. *Am J Clin Nutr*. 1975; 28:89–104. [PubMed: 803774]
- Owens IPF. Sex differences in mortality rate. *Science*. 2002; 297:2008–2009. [PubMed: 12242430]
- Pinner RW, Teutsch SM, Simonsen L, Klug LA, Graber JM, Clarke MJ, Berkelman RL. Trends in infectious diseases mortality in the United States. *JAMA*. 1996; 275:189–193. [PubMed: 8604170]
- Schuster C, Chasserot-Golaz S, Beck G. Activation of Epstein-Barr virus promoters by a growth-factor and a glucocorticoid. *FEBS Lett*. 1991; 284:82–86. [PubMed: 1647975]
- Scrimshaw NS, SanGiovanni JP. Synergism of nutrition, infection, and immunity: an overview. *Am J Clin Nutr*. 1997; 66:464S–477S. [PubMed: 9250134]
- Segerstrom SC. Social networks and immunosuppression during stress: relationship conflict or energy conservation? *Brain Behav Immun*. 2008; 22:279–284. [PubMed: 18055166]
- Shell-Duncan B. Cell-mediated immunocompetence among nomadic Turkana children. *Am J Hum Biol*. 1993; 5:225–235.

- Shell-Duncan B. Impact of seasonal variation in food availability and disease stress on the health status of nomadic Turkana children: a longitudinal analysis of morbidity, immunity, and nutritional status. *Am J Hum Biol.* 1995; 7:339–355.
- Shell-Duncan B. Evaluation of infection and nutritional status as determinants of cellular immunosuppression. *Am J Hum Biol.* 1997; 9:381–390.
- Shell-Duncan B, Wood JW. The evaluation of delayed-type hypersensitivity responsiveness and nutritional status as predictors of gastro-intestinal and acute respiratory infection: a prospective field study among traditional nomadic Kenyan children. *J Trop Pediatr.* 1997; 43:25–32. [PubMed: 9078825]
- Wander K, Brindle E, O'Connor K. Sensitivity and specificity of C-reactive protein and α_1 -acid glycoprotein for episodes of acute infection among children in Kilimanjaro, Tanzania. *Am J Hum Biol.* 2012b; 24:565–568. [PubMed: 22411080]
- Wander K, O'Connor K, Shell-Duncan B. Expanding the hygiene hypothesis: early exposure to infectious agents predicts delayed-type hypersensitivity to *Candida* among children in Kilimanjaro. *PLoS ONE.* 2012a; 7:e37406. [PubMed: 22616000]
- Washburn TC, Medearis DN, Childs B. Sex differences in susceptibility to infections. *Pediatrics.* 1965; 35:57–64. [PubMed: 14223226]
- Zaman K, Baqui AH, Yunus MD, Sack RB, Chowdhury HR, Black RE. Malnutrition, cell-mediated immune deficiency and acute upper respiratory infections in rural Bangladeshi children. *Acta Paediatr.* 1997; 86:923–927. [PubMed: 9343268]

TABLE 1

Sample characteristics

Sex		
Female	111	55.50%
Male	89	45.50%
Age in years, mean (SD)	4.83	(1.53)
Weight-for-height Z-score, mean (SD)	0.00	(0.85)
Wasted (WHZ < -2)	1	0.5%
Height-for-age Z-score, mean (SD)	-1.10	(1.28)
Stunted (HAZ < -2)	49	24.50%
Triceps skinfold in mm, mean (SD)	11.7	(3.3)
Anemia ^a	58	29.00%
Elevated CRP or AGP ^b	118	59.00%
Infectious disease diagnosis upon referral	35	17.50%
Positive DTH (induration ≥ 5 mm)	108	54.00%
Induration in mm, mean (SD)	5.8	(4.3)
Anti-Epstein-Barr virus antibody, geometric mean (AU) ^c	25,456	

^a Hemoglobin concentration under age specific cutpoints defined by WHO (Nestel, 2002).

^b C-reactive protein (CRP) or α₁-acid glycoprotein (AGP) over cutpoints defined in Wander et al., 2012b.

^c AU = arbitrary units.

TABLE 2

Univariate associations

Predictor	Outcome: positive DTH			Outcome: DTH induration			Outcome: ln EBV		
	Odds ratio	95% CI	P value	β	95% CI	P value	β	95% CI	P value
Male sex	1.08	0.62, 1.89	0.788	-0.12	-1.32, 1.09	0.849	-0.42	-0.65, -0.19	0.000
Age	1.11	0.92, 1.33	0.276	0.31	-0.08, 0.70	0.120	0.03	-0.05, 0.11	0.419
Weight-for-height Z-score	1.14	0.82, 1.59	0.432	0.54	-0.16, 1.24	0.133	-0.03	-0.17, 0.11	0.677
Height-for-age Z-score	0.87	0.70, 1.09	0.220	-0.49	-0.96, -0.03	0.039	0.05	-0.05, 0.14	0.327
Stunting	1.18	0.62, 2.27	0.612	0.48	-0.92, 1.87	0.501	-0.09	-0.37, 0.18	0.516
Triceps skinfold thickness	1.09	1.00, 1.19	0.046	0.18	0.00, 0.36	0.046	-0.02	-0.05, 0.02	0.262
Anemia	1.44	0.77, 2.67	0.251	0.96	-0.35, 2.28	0.150	0.17	-0.09, 0.43	0.192
Elevated AGP or CRP ^a	0.87	0.49, 1.53	0.620	-0.53	-1.75, 0.69	0.392	0.01	-0.23, 0.25	0.922
Infectious disease upon referral	0.50	0.24, 1.06	0.070	-1.13	-2.70, 0.44	0.159	0.09	-0.23, 0.40	0.585

^a α_1 -acid glycoprotein (AGP) or C-reactive protein (CRP); Wander et al., 2012b.

TABLE 3

Multivariate models of DTH-*Candida*

	OR	95% CI	P value
a. Logistic regression: predictors of positive DTH- <i>Candida</i>			
Male sex	1.26	0.70, 2.29	0.444
Age	1.27	1.02, 1.57	0.030
Triceps skinfold	1.16	1.02, 1.26	0.016
Height-for-age Z score	0.86	0.68, 1.08	0.198
Anemia	1.59	0.81, 3.12	0.174
Infectious disease upon referral	0.48	0.22, 1.08	0.075
	β	95% CI	P value
b. Linear regression: predictors of DTH- <i>Candida</i> induration size			
Male sex	0.19	-1.00, 1.38	0.755
Age	0.57	0.15, 0.99	0.009
Triceps skinfold	0.27	0.07, 0.47	0.008
Height-for-age Z-score	-0.49	-0.95, -0.03	0.039
Anemia	1.09	-0.23, 2.42	0.105
Infectious disease upon referral	-1.17	-2.75, 0.41	0.146

TABLE 4Multivariate models of DTH-*Candida* by triceps skinfold thickness and infectious disease

TSF under median (N = 107)			TSF over median (N = 93)				
OR	95% CI	P value	OR	95% CI	P value		
a. By triceps skinfold (TSF; median = 11.7 mm)							
Male sex	1.15	0.50, 2.60	0.745	Male sex	1.07	0.45, 2.53	0.874
Age	1.31	0.99, 1.72	0.055	Age	1.11	0.80, 1.53	0.545
Height-for-age Z	0.86	0.61, 1.27	0.515	Height-for-age Z	0.87	0.64, 1.17	0.351
Anemia	1.85	0.70, 4.88	0.215	Anemia	1.45	0.54, 3.86	0.460
ID upon referral	0.16	0.05, 0.50	0.002	ID upon referral	2.03	0.49, 8.36	0.328
ID diagnosis (N = 35)				No ID diagnosis (N = 165)			
OR	95% CI	P value	OR	95% CI	P value		
b. By infectious disease (ID) diagnosis upon referral							
Male sex	7.52	0.68, 83.48	0.100	Male sex	1.00	0.52, 1.90	0.991
Age	2.52	0.85, 7.48	0.096	Age	1.24	1.00, 1.56	0.055
Triceps skinfold	2.64	1.29, 5.42	0.008	Triceps skinfold	1.05	0.94, 1.17	0.347
Height-for-age Z	0.44	0.14, 1.36	0.152	Height-for-age Z	0.92	0.72, 1.18	0.523
Anemia	0.68	0.08, 6.05	0.726	Anemia	1.61	0.77, 3.41	0.208

TABLE 5

Multivariate model of anti-Epstein-Barr virus antibody

	β	$\exp(\beta)^a$	95% CI	<i>P</i> value
Male sex	-0.47	0.63	-0.70, -0.24	0.000
Age	0.02	1.02	-0.06, 0.10	0.613
Triceps skinfold	-0.04	0.96	-0.08, 0.00	0.061
Height-for-age Z-score	0.06	1.06	-0.03, 0.15	0.183
Anemia	0.22	1.25	-0.04, 0.48	0.098
Infectious disease upon referral	-0.01	0.99	-0.32, 0.30	0.957

^aProportional change in EBV Ab associated with each predictor.

TABLE 6

Multivariate models of EBV Ab by stunting and sex

Stunted (N = 49)				Not stunted (N = 151)			
	B	95% CI	P value		β	95% CI	P value
a. By stunting							
Male sex	-0.65	-1.08, -0.21	0.004	Male sex	-0.38	-0.67, -0.10	0.008
Age	0.07	-0.06, 0.20	0.271	Age	-0.02	-0.12, 0.09	0.771
Triceps skinfold	-0.01	-0.09, 0.06	0.745	Triceps skinfold	-0.05	-0.09, 0.00	0.057
Anemia	0.49	0.02, 0.96	0.042	Anemia	0.12	-0.19, 0.43	0.451
ID upon referral	-0.04	-0.60, 0.51	0.873	ID upon referral	0.00	-0.38, 0.38	0.996
Males (N = 89)							
Females (N = 111)							
	B	95% CI	P value		β	95% CI	P value
b. By sex							
Age	-0.02	-0.13, 0.09	0.702	Age	0.04	-0.08, 0.16	0.488
Triceps skinfold	-0.04	-0.10, 0.02	0.183	Triceps skinfold	-0.04	-0.09, 0.02	0.170
Height-for-age Z	0.12	0.01, 0.23	0.029	Height-for-age Z	-0.02	-0.17, 0.12	0.734
Anemia	0.51	0.16, 0.85	0.004	Anemia	-0.08	-0.47, 0.30	0.677
ID upon referral	-0.16	-0.56, 0.24	0.425	ID upon referral	0.21	-0.25, 0.68	0.365

TABLE 7

Expected and observed patterns in cell-mediated immune function

Predictor	Expected impact on cell-mediated immunity	DTH- <i>Candida</i>		EBV-Ab	
		Expected association	Observed association	Expected association	Observed association
Male sex	↓	↓		↑	↓ ^a
Age	↑	↑	↑ ^a	↓	
Nutrition					
Triceps skinfold	↑	↑	↑ ^a	↓	
Height-for-age	↑	↑	↓	↓	↑ ^c
Anemia	↓	↓		↑	↑ ^c
Acute infection	↓	↓	↓ ^b	↑	

^a Association consistently present and significant across models.

^b Significant interaction was present between TSF and acute infection.

^c Only observed among male children.