



Age-related patterns of cytomegalovirus antibodies accompanying Epstein-Barr virus co-infection

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Abstract

Objective: Cytomegalovirus (CMV) infection is associated with age-related chronic disease, and co-infection with Epstein-Barr virus (EBV) may compound disease risk. We aimed to assess the frequency of CMV infection and its relationship with age among EBV seropositive individuals in an Indigenous Amazonian population.

Methods: We report concentrations of CMV and EBV antibodies in dried blood spot samples collected from 157 EBV positive Shuar participants aged 15–86 years (60.5% female) to assess CMV infection rate. We used logistic and linear regression models to examine associations among CMV, EBV, and age, adjusting for sex, geographic region, and body mass index.

Results: Nearly two-thirds (63.1%) of EBV seropositive participants were also CMV seropositive. A 1-year increase in age was associated with 3.4% higher odds of CMV infection (OR [95% CI]: 1.034 [1.009–1.064], $p = .012$), but CMV antibody concentration was not significantly associated with age or EBV antibody concentration among co-infected individuals.

Conclusions: Herpesvirus-related immunosenescence may be important to understanding chronic disease risk among Shuar. Future studies should further explore the role of co-infection in shaping age-related changes in immune function.

1 | INTRODUCTION

Cytomegalovirus (CMV) infection persists throughout an individual's lifetime in a subclinical (i.e., latent) state, but immunosenescence can lead to periods of CMV reactivation, which in turn can accelerate immunosenescent processes and potentially drive a variety of downstream diseases of aging (e.g., cardiovascular disease, rheumatoid arthritis) (Aiello et al., 2017). Evidence suggests that the effect of CMV on immunosenescence may be compounded by co-infection with other herpesviruses, because co-infection is associated with greater cellular aging (Dowd et al., 2017). Co-infection with Epstein-Barr virus (EBV) is of particular interest because it is associated with higher inflammation (Bennett et al., 2012), which is a key component of immunosenescence (Franceschi et al., 2000). Although chronic inflammation is a widespread health concern in high-income settings, it is not universal across human populations (McDade et al., 2012). Therefore, studying age-related patterns of CMV and EBV antibodies in populations with low inflammation will further understanding of immunosenescence and local immune-related chronic disease risk.

Here, we investigate the relationship between CMV infection and age among EBV seropositive individuals in the Shuar, an Indigenous Amazonian population. Traditionally forager-horticulturalists, Shuar are undergoing rapid market integration (Liebert et al., 2013; Urlacher et al., 2016), have high but variable exposure to infectious disease (Blackwell et al., 2010; Urlacher et al., 2018), and no evidence of chronic low-grade inflammation (McDade et al., 2012). In accordance with evidence from high-income settings (Cannon et al., 2010), we expect that age will be positively associated with CMV seropositivity, due to cumulative exposure to CMV over time, and hypothesize that CMV exposure leads to weakened immune function. Therefore, we predict that CMV antibody concentration (CMV-Ab) will be positively associated with age among co-infected participants due to weakened immune function and a limited ability to minimize viral replication at older ages (Pourghesari et al., 2007). We also predict that CMV-Ab will be positively associated with EBV antibody concentration (EBV-Ab) while controlling for age among co-infected participants, signaling weakened immunity.

2 | METHODS

We conducted a cross-sectional study using data from EBV seropositive individuals (≥ 15 years old), collected as part of the Shuar Health and Life History Project

(SHLHP, <https://www.shuarproject.org/>) between 2008 and 2010. All lab analyses were conducted in 2015 or earlier (Eick et al., 2016; Ridgeway-Diaz, 2011). Ecuadorean Shuar number approximately 60 000–11 000 people living in over 668 communities (Liebert et al., 2013). This study included Shuar participants ($n = 160$) from 17 communities in two regions. Upano Valley communities are closer to market centers, and most residents engaged in a mixed subsistence horticulture and agro-pastoralist economy with some fishing and wage labor at the time of the study (Urlacher et al., 2016). Cross-Cutucú communities were geographically remote and relied primarily on traditional subsistence horticulture, fishing, hunting, and foraging (Urlacher et al., 2016). Because these differences can affect parasite exposure and immunity, we included a geographic region variable (Cross Cutucú vs. Upano Valley) in our analysis (Liebert et al., 2013; Urlacher et al., 2018). All study participants provided informed consent. The study was approved by the University of Oregon Institutional Review Board and authorized by the Federación Interprovincial de Centros Shuar.

Participants self-reported their age and sex, which were cross-checked against government-issued identification cards and SHLHP genealogical data (Liebert et al., 2013). Participants' height (m) and weight (kg) were measured using standard procedures, and body mass index (BMI) calculated as kg/m^2 (Liebert et al., 2013). Following prior studies, sex and BMI were included in analyses to account for their potential relationship with immunity (Bennett et al., 2012). Dried blood spot (DBS) samples were collected using standard methods (McDade et al., 2007), and the samples were stored in a -30°C freezer after field storage in a -20°C solar-powered freezer (Urlacher et al., 2018). We measured CMV-Ab and EBV-Ab using high-sensitivity enzyme-linked immunosorbent assay (ELISA) protocols (Dowd et al., 2011; Eick et al., 2016; Urlacher et al., 2018) and determined if participants were CMV seropositive (≥ 56 Au/ml) using standard guidelines for DBS samples (Dowd et al., 2011). Only EBV seropositive (> 20 Au/ml) individuals were included in the current study, and our sample is a subset from a prior study that found a high prevalence ($n = 334/337$, 99.1%) of EBV seropositivity among Shuar (Ridgeway-Diaz, 2011).

Prior to the analysis, CMV-Ab and EBV-Ab data were natural log-transformed to normalize distributions. We used Cook's distance to examine CMV-Ab data for influential outliers in the association between CMV-Ab and age. Three CMV-Ab data points ≥ 200 Au/ml had high leverage and skewed the results toward a positive association. They were excluded from the analysis. We described participants' characteristics both overall and by CMV infection status (CMV seropositive [infected] vs. CMV

seronegative [not infected]). We used multivariate logistic regression to investigate the association between age and CMV infection, adjusting for sex, region, and BMI. We used multivariate linear regression to test our predictions about CMV-Ab, age, and EBV-Ab, adjusting for sex, region, and BMI. We performed all analyses using R version 4.0.5 (R Core Team, Vienna, Austria).

3 | RESULTS

A total of 157 participants were included in the analysis, and descriptive statistics for the sample are provided in Table 1. Over half of participants ($n = 99$, 63.1%) were CMV seropositive. Participants' median (range) untransformed EBV-Ab and CMV-Ab values were 96.5 (23.1–177) Au/ml and 62.5 (27.8–160) Au/ml, respectively.

In a logistic regression model adjusted for sex, region, and BMI, a 1-year increase in age was associated with 3.4% higher odds of CMV infection (OR [95% CI]: 1.034 [1.009–1.064], $p = .012$). Among CMV infected participants ($n = 99$), CMV-Ab was not significantly associated with age in unadjusted ($\beta = 0.002$, SE = 0.002, $p = .304$) or adjusted ($\beta = 0.002$, SE = 0.002, $p = .252$) linear regression models (Figure 1A). Three individuals aged 29, 40, and 55 years had CMV-Ab ≥ 200 Au/ml and were not included in the models. These high values might suggest CMV reactivation with age, but the sample of high values is insufficient to determine if they are due to age. In the same subgroup of 99 co-infected participants, there was a significant positive association between EBV-Ab and CMV-Ab in the unadjusted linear regression model ($\beta = 0.100$, SE = 0.049, $p = .043$), but the association was

no longer significant after adjusting for age, sex, region, and BMI ($\beta = 0.089$, SE = 0.052, $p = .089$) (Figure 1B).

4 | DISCUSSION

In this cross-sectional study among 157 EBV seropositive Shuar, over half the participants were also CMV seropositive. As expected, we found that older participants had higher odds of CMV infection. Prior studies indicate that a higher seroprevalence of CMV infection among older age groups is common across populations, and this pattern can be explained by cumulative CMV exposure over time (Cannon et al., 2010). We also predicted that age would be positively associated with CMV-Ab due to age-related decreases in the immune system's ability to maintain low levels of viral replication (Pourghesari et al., 2007). However, in the subgroup of participants who were CMV seropositive, we did not find support for this prediction. Our sample included few participants older than 55 years ($n = 17$), and the small sample of older adults may have limited our ability to detect this association and examine more nuanced age effects (e.g., by decade).

CMV-EBV co-infection may be particularly important to understanding the link between latent herpesviruses and immunosenescence (Bennett et al., 2012). In our study, we found a positive association between CMV-Ab and EBV-Ab in an unadjusted model, but this relationship was no longer statistically significant in models adjusted for age. Future studies among subsistence populations in low-income settings should incorporate a broader set of immune biomarkers, such as C-reactive protein and markers of T-cell senescence, to better

TABLE 1 Participant characteristics by cytomegalovirus infection status

Characteristic	Overall ($N = 157$)	CMV negative ($n = 58$)	CMV Positive ^a ($n = 99$)
Age, years			
Median (range)	32 (15–86)	28 (15–61)	35 (15–86)
Sex			
Female	95 (60.5%)	34 (58.6%)	61 (61.6%)
Male	62 (39.5%)	24 (41.4%)	38 (38.4%)
Region			
Cross-Cutucú	35 (22.3%)	13 (22.4%)	22 (22.2%)
Upano Valley	122 (77.7%)	45 (77.6%)	77 (77.8%)
BMI, kg/m ²			
Mean (SD)	24.9 (3.19)	24.7 (3.10)	25.0 (3.25)
Missing	4 (2.5%)	1 (1.7%)	3 (3.0%)

Abbreviations: BMI, body mass index; CMV, cytomegalovirus.

^aSeropositivity determined based on a 100% specificity threshold (56 Au/ml) (Dowd et al., 2011).

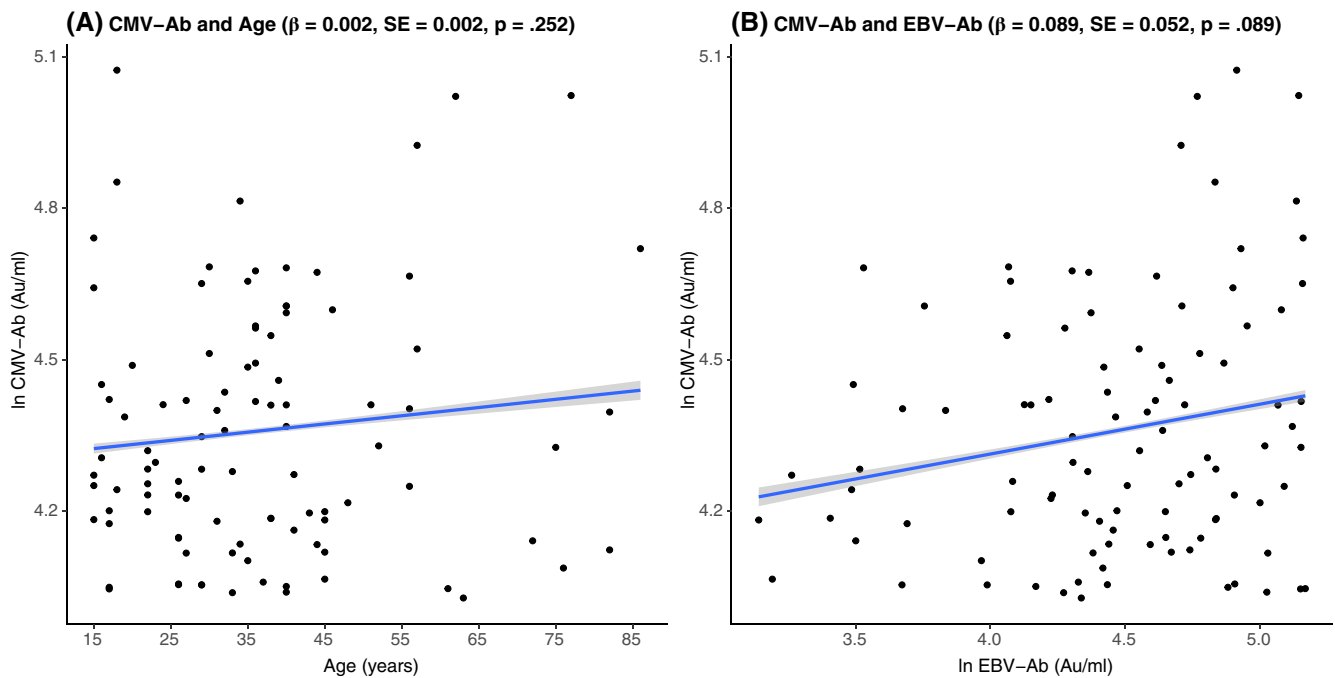


FIGURE 1 Association of cytomegalovirus antibody concentration with age and Epstein-Barr virus antibody concentration. CMV-Ab, cytomegalovirus antibody concentration; EBV-Ab, Epstein-Barr virus antibody concentration. Panel A: Linear regression model adjusted for sex, geographic region, and body mass index. Panel B: Linear regression model adjusted for age, sex, geographic region, and body mass index

understand the mechanisms through which herpesvirus infections may contribute to chronic inflammation and other processes that characterize immunosenescence (Franceschi et al., 2000).

CMV infection appears common among Shuar who are EBV seropositive, and our study suggests that older Shuar are more likely to be co-infected. This study provides a start to understanding age-related patterns of CMV infection and may help elucidate the process of immunosenescence in contexts with a high prevalence of infectious disease, low inflammation, and a shifting disease landscape related to rapid market integration.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Tyler M Barrett: Conceptualization (equal); formal analysis (lead); visualization (lead); writing – original draft (lead); writing – review and editing (equal). **Melissa A. Liebert:** Conceptualization (equal); data curation (equal); formal analysis (equal); funding acquisition (equal); investigation (equal); writing – review and editing (equal). **Geeta Eick:** Conceptualization (equal); data curation (equal); investigation (equal); methodology (equal); writing – review and editing (equal). **Julia G Ridgeway-Diaz:** Funding acquisition (equal); investigation (equal); writing – review and editing (equal). **Felicia C Madimenos:** Conceptualization (equal); formal analysis (equal); funding acquisition (equal); investigation (equal); writing – review and editing (equal). **Aaron D Blackwell:** Funding acquisition (equal); investigation (equal); writing – review and editing (equal). **Samuel S. Urlacher:** Conceptualization (equal); formal analysis (equal); funding acquisition (equal); investigation (equal); writing – review and editing (equal). **Lawrence S. Sugiyama:** Funding acquisition (equal); investigation (equal); writing – review and editing (equal). **James Josh Snodgrass:** Conceptualization (equal); formal analysis (equal); funding acquisition (equal); investigation (equal); supervision (equal); writing – review and editing (equal).

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request by emailing the form found at shuarproject.org/data-sharing to shuarproject@gmail.com. The data are not publicly available due to privacy or ethical restrictions.

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