Epstein-Barr Virus Glycoprotein Antibody Titers and Risk of Nasopharyngeal Carcinoma

Anna E. Coghill,1 Andrew McGuire,2,3,4 Sweta Sinha,1 Leah Homad,2 Irika Sinha,2 Anton Sholukh,3 Woon-Puay Koh,5,6 and Jian Min Yuan7,8

1Cancer Epidemiology Program, H. Lee Moffitt Cancer Center and Research Institute, Tampa, Florida, USA; 2Vaccine and Infectious Disease Division, Fred Hutchinson Cancer Center, Seattle, Washington, USA; 3Department of Global Health, University of Washington, Seattle, Washington, USA; 4Department of Laboratory Medicine and Pathology, University of Washington, Seattle, Washington, USA; 5Department of Laboratory Medicine and Pathology, University of Washington, Seattle, Washington, USA; 6Healthy Longevity Translational Research Programme, Yong Loo Lin School of Medicine, National University of Singapore, Singapore, Singapore; 7Agency for Science Technology and Research, Singapore Institute for Clinical Sciences, Singapore; 8Division of Cancer Control and Population Sciences, UPMC Hillman Cancer Center, Pittsburgh, Pennsylvania, USA

We evaluated antibody against Epstein-Barr virus glycoproteins (gp350, gH/gL, gB, gp42) in 97 nasopharyngeal carcinoma (NPC) cases and 97 cancer-free controls. Each unit increase in log-transformed antibody against gp350 and gH/gL was associated with 2.27 (95% confidence interval [CI], 1.20–4.29) and 2.18 (95% CI, 1.22–3.90) higher odds of NPC, respectively. This association was more apparent for NPC diagnosed within 5 years of antibody measurement.

Keywords. EBV; gH/gL; gp350; NPC.

Since the discovery in 1964 of Epstein-Barr virus (EBV) in tumor cells from a child with Burkitt lymphoma [1], EBV has been detected in epithelial tumors as well as B-cell and natural killer/T-cell lymphomas [2]. Despite this, vaccines to prevent EBV infection do not exist. Vaccine development thus far has focused on production of neutralizing antibody against EBV glycoproteins (eg, glycoprotein 350 [gp350]), with the goal of blocking the ability of EBV to infect cells [3]. This mimics the successful approach of blocking target cell infection used by both the human papillomavirus (HPV) and hepatitis B vaccines.

We previously reported that higher levels of antibody against gp350 were associated with a reduced risk of nasopharyngeal carcinoma (NPC) in Taiwanese individuals with a strong family history of cancer [4]. This provided intriguing evidence that naturally occurring immune responses to certain viral glycoproteins may be protective against development of EBV-associated disease, but those data were generated from only 18 NPC cases. Our follow-up study in the general Taiwanese population did not replicate these findings [5]. Instead, we reported significantly elevated immunoglobulin A (IgA) antibody against gp350 in 35 NPC cases (odds ratio [OR], 7.03; P < .01). This suggested that anti-EBV glycoprotein antibody levels may instead be marking individuals with elevated viremia indicative of NPC risk, much like antibody against other viral proteins such as EBV nuclear antigen (EBNA) and viral capsid antigen (VCA) [6]. In both studies, no differences by NPC case status were observed for antibody titers against glycoproteins H and L, which form a heterodimeric complex (gH/gL) required for EBV epithelial cell entry.

The objective of the current study was to evaluate antibody binding titers to 4 EBV glycoproteins in approximately 100 NPC cases from cohorts in Singapore and China, to answer the pending question of exactly what naturally occurring EBV glycoprotein antibody levels signify for NPC risk. Our analysis was focused on antibodies to (i) gp350, which mediates virion attachment to CD21/CD35; (ii) glycoprotein B (gB), which mediates the fusion of host and viral membranes; (iii) the gH/gL complex, which acts as an essential regulator of gB activity; and (iv) glycoprotein 42 (gp42), which forms a tripartite complex with gH/gL that activates gB following gp42 binding to human leukocyte antigen class II on the B-cell surface. gp42 is not required for viral entry in epithelial cells, where direct interactions between gH/gL and cellular receptors trigger gB-mediated fusion. In fact, gp42 inhibits membrane fusion of EBV with epithelial cells, and the level of gp42 associated with gH/gL on virions is thought to act as a tropsin switch.

METHODS

Serum samples were selected from the Singapore Chinese Health Study (SCHS) [7] and the Shanghai Cohort Study (SCS) [8]. The SCHS is a population-based prospective cohort drawn from permanent residents or citizens of Chinese origin who resided in government-built housing in Singapore and were 45 to 74 years of age when enrolled between April 1993 and December 1998. Blood collection began in April 1994 in a random, 3% sample of cohort participants and was expanded to the entire cohort in January 2000. A routine linkage with the Singapore Cancer Registry was performed to assess NPC diagnoses. The SCS is a prospective cohort that enrolled male
residents aged 45–64 years from Shanghai, China, between 1986 and 1989. Blood samples were collected from every participant at baseline. The cohort has been followed through annual follow-up interviews and routine linkage to the Shanghai Cancer Registry to ascertain NPC diagnoses. In total, serum was available from 97 individuals diagnosed with NPC in the 2 cohorts. For each case, 1 cancer-free individual was randomly chosen among cohort participants matched to the NPC case on age at blood draw, biological sex, neighborhood of residency, and date of blood draw.

We used a high-throughput multiplex Luminex assay to measure serum binding antibodies to recombinant EBV glycoproteins. Details of bead preparation are included in the Supplementary Methods. Serum from participants was heat-inactivated and diluted 1:50 in assay buffer (PBS plus 1% nonfat milk and 0.1% Tween-20), and 50 µL of diluted serum was added to duplicate wells that contained bead mixes of recombinant EBV glycoproteins, as well as goat antihuman IgG Fc-PE (SouthernBiotech, catalog number 2048–09) diluted 1:500 in 100 µL of assay buffer. Samples were analyzed on a Luminex LX-200 instrument, with the minimum number of each bead read set to 50. Fluorescence was translated into numerical output. Pooled sera from 10 herpes simplex virus (HSV) type 2–seropositive healthy donors, as well as serum from healthy donors confirmed seronegative for HSV-1 and HSV-2, were included on each plate to ensure consistency across assay runs (plate control). The anti-EBV glycoprotein antibody level for each participant was divided by the output of the plate control to create standardized output for statistical analyses.

We compared the log-transformed, standardized output for each anti-EBV glycoprotein antibody between individuals who did versus did not develop NPC using a nonparametric Mann-Whitney test. We further compared output between cancer-free controls and NPC cases stratified by time between baseline blood draw and NPC diagnosis, including <5 years, 5–10 years, and >10 years. Finally, for 122 participants (60 cases and 62 controls) with data on both age at blood collection and biological sex, we examined the association between anti-EBV glycoprotein antibody and NPC risk using logistic regression adjusted for both age at blood draw and biological sex. Approval to test and analyze data generated from existing biospecimens was granted by Moffitt Cancer Center.

RESULTS

The average age of the 194 participants selected for this study was 55 years, consistent across study group due to the matched study design. Likewise, 72% to 74% of participants across both groups were males. Among the 97 individuals diagnosed with NPC, the distribution of time between blood draw and NPC diagnosis was as follows: <5 years (29%), 5–10 years (37%), and >10 years (34%). For the 28 NPC patients diagnosed within 5 years of blood draw, the average time between blood draw and receipt of this diagnosis was 2.3 years (standard deviation, 1.3 years).

The levels of both anti-gp350 and anti-gH/gL IgG antibody were elevated among individuals diagnosed with NPC compared to cancer-free controls (P = .08 and P < .01, respectively). No evidence of an association with NPC was observed for binding antibody against gB (P = .92) or gp42 (P = .60). As illustrated in Figure 1, this association was not equivalent when analyzed according to lag time between blood draw and NPC diagnosis. Among NPC cases, IgG antibody level was highest in those with an imminent NPC diagnosis (lag time <5 years) and lowest in those with a NPC lag time >10 years (P_{trend} < .01 for gp350 and gH/gL). Both gp350 (P < .01) and gH/gL (P < .001) levels in NPC cases with a lag time <5 years were significantly higher than those in cancer-free controls. In contrast, no statistically significant differences were observed between NPC-free controls and cases whose blood was drawn an extended period (>5 years) prior to NPC diagnosis.

These patterns were confirmed in regression analyses adjusted for age and sex. Specifically, for each unit increase in the standardized, log-transformed anti-EBV gp350 or gH/gL IgG output, individuals were 2.27 (95% confidence interval [CI], 1.20–4.29) and 2.18 (95% CI, 1.22–3.90) times more likely to be diagnosed with NPC, respectively. Associations were not observed for gB (OR, 0.94 [95% CI, .51–1.73]) or gp42 (OR, 1.01 [95% CI, .49–2.09]). The statistically significant associations for anti-gp350 and anti-gH/gL were due to elevated antibody levels in those with an imminent diagnosis (ie, NPC <5 years).

Figure 1. Antibody output by study group. (Log) anti–Epstein-Barr virus antibody level (y-axis) according to time between blood draw and nasopharyngeal carcinoma (NPC) diagnosis (>10 years, 5–10 years, <5 years; x-axis) and NPC-free controls (Cont.; x-axis) for each glycoprotein. Antibody differences between group were evaluated using a nonparametric Mann-Whitney test. Abbreviations: MFI, median fluorescence intensity; NPC, nasopharyngeal carcinoma.
Removing NPC cases diagnosed within 5 years resulted in associations that were no longer statistically significant (anti-gp350: OR, 2.03 [95% CI, .73–5.62]; anti-gH/gL: OR, 1.72 [95% CI, .95–3.13]).

**DISCUSSION**

Our data represent the largest assessment to date of the association between naturally occurring anti-EBV glycoprotein antibody levels and NPC risk. Results suggest that individuals with elevated anti-gp350 or -gH/gL IgG levels are more likely to be diagnosed with NPC, and this elevated cancer risk is specific to NPC diagnosed within 5 years of blood draw.

The clear increase in anti-glycoprotein IgG antibody close to the time of NPC diagnosis is a general feature of an effective disease biomarker. The observed pattern for anti-EBV gp350 and gH/gL IgG antibodies is comparable to other high-risk NPC biomarkers like IgA antibody against EBNA or VCA viral proteins. EBNA and VCA IgA are currently being evaluated in southeast China as early detection biomarkers for NPC [6]. The most recent data provide evidence that use of EBNA and VCA IgA levels as biomarkers to screen for NPC (ie, detect disease early) may have the potential to decrease NPC mortality when applied as a population-based testing tool [9]. We propose that future efforts should utilize samples from population-based cohorts to examine whether addition of anti-EBV glycoprotein binding titers could improve NPC prediction beyond current anti-EBV IgA biomarkers. Of note, the addition of biomarkers to current NPC screening algorithms should prioritize specificity to reduce rates false positivity test rates. It is possible that the relatively frequent positivity against anti-EBV glycoprotein antibody observed in the general population in Taiwan will preclude a high level of NPC case discrimination for this marker.

These data were generated using 97 prospectively identified NPC cases from general population cohorts in Singapore and Shanghai and support our prior work from the general population cohorts in Singapore and Shanghai and support our prior work from the general population cohorts in Singapore and Shanghai. In that prior study of 35 cases, we also observed an association between higher anti-EBV glycoprotein antibody and NPC risk. This contrasts with inverse findings using 18 cases from a cohort of Taiwanese individuals with a strong family history of NPC (ie, multiple first- or second-degree family members with NPC) [4]. It is plausible that EBV-based biomarkers perform differently in populations with varying underlying disease risk. Additionally, the characteristics of the anti-glycoprotein antibody must be considered. In the study of those with a strong family history of NPC, associations were examined between both total anti-gp350 antibody and the subset of antibody with a demonstrated ability to neutralize, or block, EBV infection. In our current study, we limited our examination to total anti-glycoprotein antibody.

Importantly, the lack of an inverse association between naturally occurring levels of anti-EBV glycoprotein antibody and NPC does not imply that vaccine strategies targeting these EBV proteins are flawed. The approach of producing antibody that can neutralize infection has achieved great success for preventing HPV infections that lead to HPV-associated cancers. It should be noted that the HPV vaccine does not rely on naturally occurring levels of antiviral antibody to be effective but instead uses a virus-like particle injection to produce a high level of antibody to achieve ubiquitous neutralization. In line with this notion, we and others have developed self-assembling nanoparticle vaccine candidates based on the gH/gL and gp350 proteins that elicit high levels of neutralizing antibodies in preclinical animal models [3, 10]. In addition, it must be acknowledged that although our data support the observation that anti-EBV glycoprotein antibody levels are associated with NPC risk, the absolute risk of NPC remains quite low on a population level in most regions globally.

In summary, our data suggest that individuals with elevated anti-gp350 or anti-gH/gL IgG levels are more likely to be diagnosed with NPC in the next 5 years. This suggests that naturally occurring anti-glycoprotein antibody in adults with lifelong EBV infection marks poor EBV control and exposure to elevated viremia.

**Supplementary Data**

Supplementary materials are available at Open Forum Infectious Diseases online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

**Notes**

Patient consent. This analysis was approved by the Moffitt Scientific Review Committee and Advarra Institutional Review Board. Patient consent was not required as the data used were previously collected for research purposes; this study involved no additional patient contact.

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