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High Levels of Antibody that Neutralize B-cell Infection of Epstein–Barr Virus and that Bind EBV gp350 Are Associated with a Lower Risk of Nasopharyngeal Carcinoma

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Abstract

Purpose: Elevated IgA antibodies indicative of ongoing exposure to Epstein–Barr virus (EBV) are high-risk biomarkers for nasopharyngeal carcinoma (NPC), an EBV-related epithelial tumor. However, protective biomarkers that limit exposure to the virus have not been defined. We evaluated whether antibodies that can neutralize EBV infection by targeting glycoproteins involved in viral cell entry, including EBV vaccine candidate glycoprotein 350 (gp350), were associated with lower NPC risk.

Experimental Design: In a prospective cohort of 2,557 individuals from 358 high-risk NPC multiplex families in Taiwan, we identified 21 incident NPC cases and 50 disease-free controls. To complement data from high-risk families, we further identified 30 prevalent NPC cases and 50 healthy controls from the general Taiwanese population. We quantified EBV-neutralizing antibody, antibodies against EBV glycoproteins involved in B-cell and epithelial cell entry, and anti-EBNA1 IgA, a high-risk NPC biomarker.

Results: EBV-neutralizing antibodies blocking B-cell infection and anti-gp350 antibodies were present at significantly higher levels in disease-free controls compared with incident NPC cases ($P < 0.03$). Family members with both low EBV-neutralizing potential and elevated EBNA1 IgA had a 7-fold increased risk of NPC (95% CI, 1.9–28.7). Neutralizing antibodies against epithelial cell infection did not differ between incident cases and disease-free controls. Anti-glycoprotein antibody levels measured at diagnosis (prevalent NPC) were significantly higher than levels measured prior to diagnosis ($P < 0.01$).

Conclusions: Elevated titers of EBV-neutralizing antibody and anti-gp350 antibody were low-risk biomarkers for NPC. These data suggest that a vaccine that induces potent EBV gp350 and B-cell–neutralizing antibodies could reduce the risk of EBV-related cancers such as NPC. *Clin Cancer Res; 22(14); 3451–7. ©2016 AACR.*

Introduction

The development of anaplastic nasopharyngeal carcinoma (NPC) is hypothesized to be a multifactorial process, involving exposure to environmental risk factors, inherited genetic susceptibility, and infection with the essential cofactor, Epstein–Barr virus (EBV; refs. 1–5). Although this epithelial tumor is rare in the United States, anaplastic NPC occurs at high rates in certain geographic regions such as Southeast Asia (6, 7). Elevated antibody titers against EBV proteins have been linked to the risk of NPC. Specifically, IgA antibodies indicative of exposure to EBV at mucosal surfaces, likely due to reactivation of EBV from latency in B cells, are elevated years prior to NPC diagnosis and have been evaluated for their utility as screening biomarkers (8–11). Despite the identification of high-risk biomarkers for NPC, no parallel antibody markers identifying those at lower risk of disease have been defined. Such low-risk markers could provide insight into immune responses that control EBV infection and could potentially be targeted by vaccines for the prevention of EBV-related malignancies.

One logical candidate for such low-risk markers for NPC is antibody produced against glycoproteins involved in viral entry. EBV produces envelope glycoproteins that recognize and bind surface proteins on B cells and epithelial cells, facilitating EBV cell entry. Of particular interest is the EBV glycoprotein 350 (gp350) that binds CD21 and allows EBV to enter B cells, where it can establish a latent infection (reviewed by Longenecker and colleagues, 2013; ref.12). Antibodies produced against this glycoprotein could bind and therefore block the interaction

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Translational Relevance

Elevated IgA antibodies against Epstein–Barr virus (EBV) proteins (i.e., EBNA1) are high-risk biomarkers for nasopharyngeal carcinoma (NPC), but protective antibodies that could inform NPC prevention and further EBV vaccine efforts have not been identified. Antibodies targeting glycoproteins involved in EBV cell entry can neutralize infection and are logical candidates for such low-risk biomarkers. A glycoprotein 350 (gp350) vaccine tested in young adults successfully elicited EBV-neutralizing antibodies and demonstrated efficacy against EBV-related infectious mononucleosis. However, no human studies to date have prospectively evaluated EBV-neutralizing or anti-gp350 antibodies in relation to the development of EBV-related cancer. We report here for the first time that elevated titers of EBV-neutralizing and anti-gp350 antibodies are associated with a lower risk of developing NPC. These results suggest that development of a vaccine that induces potent EBV-neutralizing activity might reduce the burden of EBV-related cancers.

with its B-cell receptor, neutralizing viral entry (13, 14). In fact, we have previously demonstrated using a GFP-based infection neutralization assay that the antibody titer against EBVgp350 correlates with the ability to neutralize EBV infection of B cells in healthy U.S. blood donors (15).

gp350 has been evaluated as an EBV vaccine candidate in both nonhuman primates and humans (reviewed by Cohen, 2015; ref.16), and gp350 vaccines elicit both anti-gp350 and EBV-neutralizing antibody in humans (17, 18). One phase II trial of recombinant gp350 demonstrated vaccine efficacy against developing the EBV-related disease infectious mononucleosis in young adults (19). However, no human studies have prospectively evaluated the relationship between EBV-neutralizing antibody or anti-gp350 antibody and the risk of an EBV-related epithelial tumor such as NPC. Lessons may be borrowed from studies of neutralizing antibodies against other oncogenic pathogens. Higher levels of naturally occurring anti-L1 antibody against oncogenic HPV-16 and HPV-18 have been linked to lower risk of subsequent HPV-16 and HPV-18 infection (20), which is important given that the successful HPV vaccine induces high levels of anti-L1–neutralizing antibody to prevent HPV infection and the development of cervical and other HPV-related preneoplastic lesions (21–23). Likewise, protection against the carriage of hepatitis B (HBV) among vaccinated children is related to the production of neutralizing antibody against HBV surface antigen (24), and vaccination is related to decreasing rates of liver cancer (25).

To determine whether neutralizing antibodies to EBV infection are associated with a reduced risk of developing NPC, we compared the level of neutralizing antibody that prevents EBV infection of both B cells and epithelial cells among NPC cases and disease-free controls.

Materials and Methods

We evaluated the association between neutralizing antibodies against EBV and the risk of developing NPC in the Taiwan Family Study (TFS). The TFS is a prospective cohort of individuals recruited between 1996 and 2005 from 358 NPC multiplex families (i.e., >2 family members affected by NPC), a high-risk population with NPC rates approximately 100-fold higher than the incidence in the United States (10, 26). Of relevance to this report, 2,557 disease-free family members had blood drawn at study enrollment and have been followed for the development of incident NPC. As described previously, both passive (linkage to national tumor registry) and active (clinical evaluation) methods were used to ascertain incident NPC. The TFS was approved by the Office of Human Subjects Research at the NCI (Rockville, MD).

Case selection

Incident cases ascertained through December 31, 2010, were selected ($N = 21$). The average time between baseline blood draw and NPC diagnosis among incident cases was 5.4 years (median = 4.9; SD = 3.0). Individuals who did not develop NPC as of December 31, 2010 (i.e., disease-free controls), were also selected ($N = 50$) and frequency matched to cases on gender and age (5-year intervals). Cases and controls were not matched within the same family so that findings could be applied broadly to multiplex families. In addition to measuring the level of neutralizing antibody in blood drawn at baseline, we measured antibodies to EBV gp350 and gH/gL in the following cases and controls selected randomly from participants in a previous case-control study conducted by our group in Taiwan with sufficient sample volume to complete testing: (i) blood drawn at the time of NPC diagnosis for 30 prevalent, early-stage NPC cases and (ii) blood drawn from 50 community controls (27).

EBV neutralization assays

Baseline serum was tested for neutralization of EBV infection of B cells using an assay that has previously been demonstrated to correlate with the inhibition of B-cell transformation in healthy U.S. blood donors (15). Neutralization of EBV infection of epithelial cells was performed using a similar assay. Serum from study participants, mAb, or media was serially diluted in 2-fold steps, and 25 μL of each diluted serum sample was incubated for 2 hours with EBV-expressing GFP. This mixture was added to Raji B cells or SVKCR2 epithelial cells in 96-well plates and incubated for 3 days at 37°C. Cells were resuspended in PBS, fixed in 2% paraformaldehyde in PBS, and GFP-positive cells were quantified using an Accuri C6 Flow Cytometer (BD Biosciences) and BD CSampler Software to quantify the neutralization of EBV–GFP infection. The neutralization assays were conducted using type I EBV (B95.8 and Akata strains for B cells and epithelial cells, respectively).

The dilution of serum that inhibited infectivity of EBV by 50% (IC_{50}), based on the reduction of the number of GFP-positive cells, was calculated by nonlinear regression using GraphPad Prism software. Neutralizing activity was considered absent when the software program failed to fit the results to an appropriate regression curve. A known EBV-positive serum sample was tested each day the assay was performed, and statistical analyses were conducted using a standardized $IC_{50,}$ defined as the IC_{50} for each study participant divided by the IC_{50} of the EBV-positive sample tested on the same day.

EBV gp350 and gH/gL antibody assays

In addition to measuring B-cell and epithelial cell neutralization, baseline serum was also tested for antibodies specific to EBV gp350 and gH/gL. The interaction between EBVgp350 and

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CD21 on B cells is crucial for initiating entry into EBV B cells, and gH and gL are part of not only the fusion complex required for EBV B-cell entry after gp350 binding but also facilitate entry into epithelial cells via binding to integrins (28).

We measured anti-EBV gp350 and gH/gL using luciferase immunoprecipitation (LIPS) assays (ref.15 and Bu and colleagues, unpublished data). Cell lysates containing EBV glycoprotein–*Renilla* luciferase fusion proteins were incubated with serum from study participants, immunoprecipitated with protein A/G beads, and incubated with coelenterazine substrate. Light units were quantified using a luminometer to obtain a quantitative measure of the antibody titer in each sample. Tests were conducted in triplicate, and statistical analyses were performed using a standardized value, defined as the average antibody titer of the triplicate tests, divided by a plate-specific cut-off value. This plate-specific cut-off value was defined by calculating the mean + 2 times the SD of the antibody titer from 4 known EBV-negative sera.

EBV EBNA1 antibody assay

Baseline serum from study participants was previously tested for IgA antibodies against a synthetic EBNA1 peptide using an ELISA (11). Analyses were conducted using the mean of duplicate absorbance values for each sample, divided by a plate-specific cut-off value. Two known EBV IgG/IgA–positive reference sera were tested at 1:100, and plate-specific cut-off value was defined by calculating the mean OD450 reactivity + 2 times the SD of 4 defined EBV-negative sera (1:100) tested in duplicate.

Assay reproducibility

To determine assay reproducibility, we compared results from 35 and 13 duplicate samples for the LIPS and GFP-based neutralization assays, respectively, performed by laboratory personnel blinded to the identity of the samples. We observed highly statistically significant correlations between blinded duplicate measures for the LIPS (gp350: Pearson = 0.85, Spearman = 0.75; gH/gL: Pearson = 0.96, Spearman = 0.95) and neutralization assays (B cell: Pearson = 0.92, Spearman = 0.91; epithelial cell: Pearson = 0.82, Spearman = 0.93).

Statistical analyses

We report the mean IC_{50} (i.e., serum titer required for 50% neutralization of EBV infection) between incident cases and disease-free controls for both B cells and epithelial cells. Results for *t* tests comparing these means according to NPC status were similar when based on raw or standardized data; statistical tests reported in the text are based on standardized data. We further report the mean serum anti-gp350 and anti-gH/gL antibody titers between members of NPC multiplex families who did or did not develop NPC during follow-up. All differences were evaluated using an unpaired, two-sample Welch–Satterthwaite *t* test that did not assume equal variance, with an $\alpha < 0.05$ considered to be statistically significant. All *t* tests were conducted using SAS version 9.3.

Results

We evaluated serum obtained before the diagnosis of cancer from 21 incidentally detected NPC cases (18 diagnosed >1 year after baseline blood draw) and serum from 50 age and gender matched controls selected from unaffected individuals in our NPC multiplex family study. The matching was effective, with a similar distribution of males to females (76% vs. 24%) and a similar average age (approximately 43 years) among both incident cases and disease-free controls (Table 1). Of the 21 incident NPC cases, 13 (61.9%) reported a history of smoking, with 10 of the 13 reporting current smoking at the time of blood draw. Nineteen of the 21 incident NPC cases had available information on cancer stage at diagnosis, with an equal distribution of early (stage I–II) and advanced (stage III–IV) disease observed (stage I, 21.1%; stage II, 26.3%; stage III, 21.1%; Stage IV, 26.3%). Each incident NPC diagnosis with recorded histologic data ($N = 16$) was classified as "type 2" disease (type IIa, $N = 10$; type IIb, $N = 6$). Serum obtained after NPC diagnosis from prevalent cases and community controls selected from a previously conducted case–control study in Taiwan were also evaluated.

Individuals who develop NPC have reduced levels of neutralizing antibody that prevents EBV infection of B cells, but not infection of epithelial cells

Serum from incident NPC cases had lower titers of neutralizing antibody against EBV infection of B cells (raw $IC_{50} = 93.98$; standardized $IC_{50} = 0.07$) compared with serum from persons who remained disease-free during follow-up (raw $IC_{50} = 110.5$; standardized $IC_{50} = 0.09$). This difference was statistically significant when incident NPC cases diagnosed >1 year after baseline blood draw (raw $IC_{50} = 47.95$; standardized $IC_{50} = 0.04$) were compared with disease-free controls ($P = 0.03$; Fig. 1) We observed no difference in the level of neutralizing antibody

Table 1. Characteristics of study participants, according to NPC status

	Prospective study of high-risk, NPC multiplex families		Case–control study of prevalent NPC	
	Incident NPC ($N = 21$)	Disease-free controls ($N = 50$)	Early-stage NPC ($N = 30$)	Controls ($N = 50$)
Age (years)				
21–34	4 (19.1)	12 (24.0)	7 (23.3)	5 (10.0)
35–39	5 (23.8)	9 (18.0)	5 (16.7)	10 (20.0)
40–54	9 (42.9)	16 (32.0)	11 (36.7)	23 (46.0)
55–74	3 (14.3)	13 (26.0)	7 (23.3)	12 (24.0)
Sex				
Female	5 (23.8)	12 (24.0)	8 (26.7)	17 (34.0)
Male	16 (76.2)	38 (76.0)	22 (73.3)	33 (66.0)
EBNA1 IgA positive[a]	17 (85%)[b]	24 (48%)	30 (100%)	25 (50%)

[a]Seropositivity for EBNA1 IgA calculated as described previously (11).
[b]Seropositivity for EBNA1 IgA calculated out of 20 incident NPC cases with available data.

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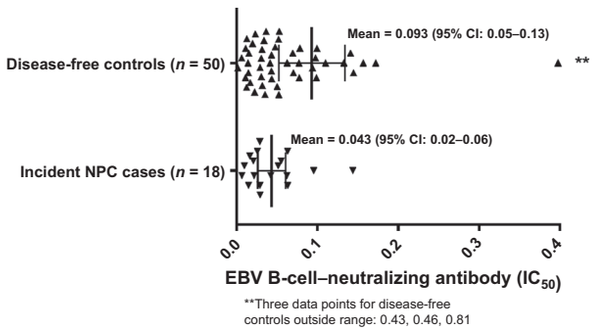

Figure 1.
Difference in neutralizing antibodies against B-cell infection between disease-free controls and incident NPC cases.

against EBV infection of epithelial cells in persons diagnosed with incident NPC compared with disease-free controls (Fig. 2).

Neutralization of B-cell infection was significantly correlated with the anti-gp350 antibody titer in our study participants (Pearson = 0.70, Spearman = 0.51; $P < 0.01$; Supplementary Fig. S1). The gH/gL complex is involved in both epithelial and B-cell entry, and anti-gH/gL titer was accordingly correlated with neutralization of EBV entry into both epithelial cells (Pearson = 0.43; Spearman = 0.77; $P < 0.01$) and B cells (Pearson = 0.54; Spearman = 0.73; $P < 0.01$; Supplementary Fig. S2 and S3).

Individuals who develop NPC have reduced levels of antibody against EBV gp350, but not EBV gH/gL

EBV gp350 is the major target for neutralizing antibodies against EBV B-cell infection (13, 14). Consistent with our neutralization data, mean anti-gp350 antibody levels measured at baseline were significantly lower ($P = 0.05$) in individuals who developed NPC during follow-up (x = 24.10) compared with persons who remained disease free (x = 53.48). Mean anti-gp350 antibody levels were particularly low (x = 19.45) among incident cases diagnosed >1 year after baseline blood draw compared with disease-free controls ($P = 0.02$; Fig. 3)

EBV replicates in epithelial cells of the oropharynx and is shed in saliva (29, 30); the virus also establishes a latent infection in epithelial cells of premalignant lesions of the nasopharynx (31). EBV entry into epithelial cells is thought to be mediated through gH/gL binding to integrins (32), and the gH/gL complex is required for EBV attachment and entry into epithelial cells (33, 34). Nonetheless, the mean anti-gH/gL antibody level measured at baseline was not significantly lower ($P = 0.21$) in persons who developed NPC during follow-up (x = 9.54) compared with disease-free controls (x = 12.21; Fig. 4). The correlation between anti-gp350 antibody (associated with B-cell infection) and anti-gH/gL antibody (associated with both epithelial and B-cell infection) was significant but of moderate magnitude (Pearson = 0.24; Spearman = 0.47; $P < 0.01$), indicating that these immune responses to EBV are only partially related (Supplementary Fig. S4).

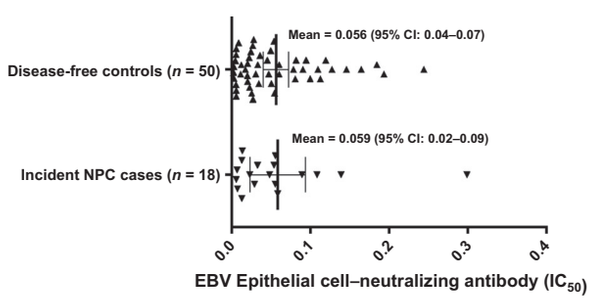

Figure 2.
Difference in neutralizing antibodies against epithelial cell infection between disease-free controls and incident NPC cases.

The risk of incident NPC is highest among persons with both elevated levels of EBNA1 IgA antibody and low levels of gp350 antibody

IgA antibody against the EBNA1 protein is an established high-risk biomarker for NPC (11). We measured EBNA1 IgA in our study participants and confirmed that individuals positive for EBNA1 IgA were significantly more likely to develop incident NPC [risk ratio = 6.1; 95% confidence interval (CI), 1.6–23.6]. We observed no significant correlation between EBNA1 IgA and anti-gp350 antibody in this population (Pearson = −0.05, Spearman = 0.07; $P > 0.60$).

We next combined the anti-EBNA1 IgA and anti-gp350 antibody titers into a single composite measure and observed that the association of EBNA1 IgA titer with future NPC risk was significantly different according to participant gp350 level ($P_{interaction} = 0.02$). Compared with EBNA1 IgA–negative individuals, those who were EBNA1 IgA–positive and had high levels of anti-gp350 antibody (i.e., high neutralizing potential) had a 1.7-fold increased risk of NPC (95% CI, 0.15–20.2). Notably, EBNA1 IgA–positive individuals with low levels of anti-gp350 antibody (i.e., low neutralizing potential) had a 7.3-fold increased risk of NPC (95% CI, 1.9–28.7; $P_{trend} < 0.01$; Table 2). This risk pattern was also observed when combining EBNA1 IgA with total neutralizing antibody against B-cell infection (EBNA1 IgA–positive/high neutralizing potential: OR = 4.7; EBNA1 IgA–positive/low neutralizing potential: OR = 7.3; $P_{trend} < 0.01$).

Antibodies to both EBV gp350 and EBV gH/gL are elevated once NPC is present

Anti-gp350 and anti-gH/gL antibodies measured in blood drawn at the time of diagnosis of NPC (i.e., prevalent disease) were significantly higher than levels in both population-based controls and in the baseline samples taken before the diagnosis of incident NPC ($P < 0.01$; Supplementary Fig. S5 and S6).

Discussion

We report evidence that neutralizing antibodies against EBV infection are related to the risk of developing an EBV-related malignancy. Specifically, lower titers of antibody against EBV gp350, a viral glycoprotein important for CD21-mediated B-cell entry, were associated with higher NPC risk. These data provide strong evidence that higher neutralizing activity in patient serum against B-cell infection is inversely correlated with the development of NPC. No substantial difference in neutralizing antibodies against epithelial cell infection, as well as antibodies against gH/gL, were observed in patient sera collected before NPC diagnosis.

A gp350-based vaccine was first evaluated for protection against EBV-related malignancies in the 1980s, with vaccine efficacy

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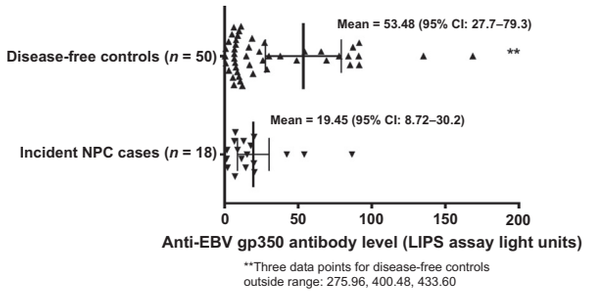

Figure 3.
Difference in anti-gp350 antibodies between disease-free controls and incident NPC cases.

observed against the development of lymphomas among cottontop tamarins (35). The first human study of a gp350 vaccine used recombinant vaccinia virus expressing gp350 and found a trend toward reduced EBV infection in seronegative children who received the vaccine (17). A phase I/II trial demonstrated that a recombinant gp350 vaccine in adjuvant induced the production of anti-gp350 and B-cell–neutralizing antibodies (18). More importantly, a 3-dose regimen of this gp350 vaccine in adjuvant among EBV-seronegative young adults ($N = 181$) demonstrated efficacy against the development of infectious mononucleosis (vaccine efficacy = 78%), although not against EBV infection (vaccine efficacy = 27%; ref.19). This result suggested that although the vaccine might not provide sterilizing immunity, it may nonetheless be useful in preventing EBV-related diseases, consistent with our findings that elevated anti-gp350 antibody levels confer a lower risk of NPC in a high-risk population of mostly EBV-seropositive adults.

We postulate that the association of anti-gp350 antibody and B-cell–neutralizing antibody with a reduction in NPC risk is due to the ability of these antibodies to block ongoing viral spread, thereby limiting reactivation of EBV in lymphocytes over the course of a lifetime. A previous study in nonhuman primates showed that rhesus gp350–vaccinated animals with higher levels of anti-gp350 antibody were not always protected from rhesus EBV primary infection compared with controls but did have lower levels of EBV DNA in the blood years after infection (36). As EBV-infected B cells are thought to traffic to the oropharynx where the virus is shed, anti-gp350 and B-cell–neutralizing antibodies may lessen an individual's cumulative exposure to EBV at mucosal sites including the nasopharynx, thereby reducing the risk of developing NPC. Furthermore, infection of B cells *in vitro* with EBV results in a large percentage of virus remaining on the surface of the cells, and this virus can efficiently infect epithelial cells (37); thus, antibodies that neutralize B-cell infection might reduce transfer of EBV from B cells to epithelial cells.

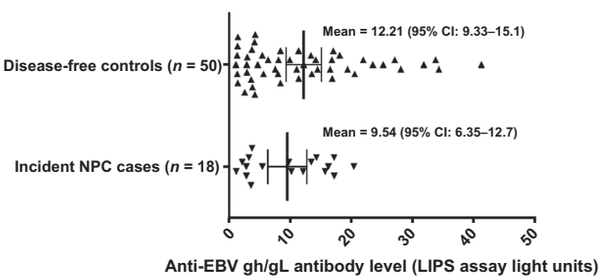

Figure 4.
Difference in anti-gH/gL antibodies between disease-free controls and incident NPC cases.

Our prospective design involved examining immune responses to EBV prior to the development of disease and has implications for disease prevention. This complements prior cross-sectional studies that evaluated anti-gp350 antibody as a diagnostic rather than a predictive tool for NPC. IgG/IgA antibodies measured cross-sectionally were elevated in blood drawn from prevalent NPC cases compared with healthy individuals (38, 39), consistent with our observations. This pattern was further supported by our observation of higher anti-gp350 titers in 3 patients with blood drawn within approximately one year of NPC diagnosis, compared with anti-gp350 titers in 18 incident NPC cases with blood drawn an average of 5 years prior to disease. Caution is warranted when considering anti-gp350 antibody as a one-time clinical screen; individuals with higher anti-gp350 antibody titers could either be less likely to develop NPC if screening was conducted years prior to disease, or they could represent prevalent NPC cases if screening was conducted after NPC was present.

We observed no significant differences in neutralizing antibodies against epithelial cell infection between individuals who did or did not develop NPC. Instead, our data suggest that blocking EBV infection of B cells is more important as a predictive marker for this EBV-related epithelial tumor. B cells are the primary target for EBV and the source of the latent EBV pool in humans. Although the difference was not statistically significant, anti-gH/gL antibody titer was marginally higher in disease-free controls compared with incident NPC cases, possibly reflecting the involvement of gH/gL in EBV entry into both B cells and epithelial cells. Finally, it should be noted that the stronger association observed between NPC and anti-gp350, relative to anti-gH/gL, may be due to additional antibody functions beyond the neutralization of EBV B-cell infection. Some studies of gp350 vaccines in cottontop tamarins reported a lack of correlation between vaccine-induced EBV-neutralizing antibody and protection against EBV-related lymphomas, suggesting that other immune responses may be important (40). For example, anti-gp350 antibodies in human sera may have a role in antibody-dependent cellular cytotoxcitiy (39, 41).

Our findings have implications for EBV-related malignancies in addition to NPC. For example, the robust immune response to EBV that occurs when the virus is acquired later in life (i.e., in adolescence; ref.42) is often accompanied by infectious mononucleosis, a condition associated with a higher risk of developing Hodgkin lymphoma (43). Infectious mononucleosis is not associated with an increased risk of NPC (44, 45). In fact, certain epidemiologic data actually suggest that earlier age at infection during childhood, as opposed to delayed infection, is a risk factor for NPC development (46). However, in both instances, regardless of the mechanism by which EBV contributes to disease, a vaccine that effectively controls the initial and/or subsequent persistent infection might decrease the likelihood of developing EBV-associated cancer.

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Table 2. NPC risk, according to a combined measure of anti-EBNA1 IgA and anti-gp350 antibodies

	Total (*N*)	Incident case (*N*)	Disease-free control (*N*)	OR (95% CI)
EBNA1 IgA negative[a]	29	3	26	1.00 (referent)
EBNA1 IgA positive	41	17	24	6.1 (1.6–23.6)
Elevated anti-gp350[b]	6	1	5	1.7 (0.15–20.2)
Not elevated anti-gp350	35	16	19	7.3 (1.9–28.7)

[a]Seropositivity for EBNA1 IgA defined as defined previously (11).
[b]Elevated anti-gp350 antibody level defined as upper median of the anti-gp350 distribution in participants.

Strengths of our study include the use of multiple assays to measure antibodies that block B-cell and epithelial cell infection. The sample size was limited due to the low number of incident NPC cases. However, the magnitude of the association observed was large, and the consistent data from separate assays measuring EBV neutralization of B-cell infection and EBV gp350 increase confidence in our conclusions. Notably, the significant correlation between the more labor-intensive neutralization assay and the more affordable and higher throughput LIPS assay suggests that the latter assay reflects neutralization activity and can be applied in large-scale studies of EBV-related disease. This study was embedded in what is to our knowledge the only registry-based family study of NPC, a unique resource for studying this rare outcome in a prospective setting. The combination of low-risk marker data with existing EBNA1 IgA data from our study shows promise for understanding the role of neutralizing antibodies in the natural history of EBV-related disease among populations with documented elevations in high-risk biomarkers. Importantly, the prospective, inverse association that we observed between anti-gp350 antibodies and familial NPC needs to be replicated in a study of sporadic NPC from an endemic region to provide additional evidence that our data from the multiplex family setting of high genetic predisposition could translate to the general population.

In summary, higher levels of anti-gp350 antibody and neutralizing antibody that can block EBV infection of B cells were associated with a lower risk of developing NPC in a high-risk adult population in Taiwan. Importantly, this represents the first low-risk biomarker of NPC as a complement to the well-established high-risk antibodies such as anti-EBNA1 IgA. Identification of low-risk markers for malignancies may have important implications for prevention strategies. Our findings suggest that development of a vaccine that induces potent EBV gp350 and B-cell–neutralizing antibodies might reduce the risk of EBV-related diseases such as NPC.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed

Authors' Contributions

Conception and design: A.E. Coghill, W. Bu, A. Hildesheim, J.I. Cohen
Development of methodology: W. Bu, H. Nguyen
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): W. Bu, W.-L. Hsu, K.J. Yu, P.-J. Lou, C.-P. Wang, C.-J. Chen, A. Hildesheim
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): A.E. Coghill, W. Bu, C.-P. Wang, C.-J. Chen, A. Hildesheim, J.I. Cohen
Writing, review, and/or revision of the manuscript: A.E. Coghill, W. Bu, P.-J. Lou, C.-P. Wang, C.-J. Chen, A. Hildesheim, J.I. Cohen
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): H. Nguyen, W.-L. Hsu, A. Hildesheim
Study supervision: W. Bu, P.-J. Lou, C.-J. Chen, A. Hildesheim, J.I. Cohen

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