Epstein-Barr Virus Antibodies and the Risk of Associated Malignancies: Review of the Literature

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Epstein-Barr virus (EBV), a ubiquitous herpes virus that infects 90% of humans by adulthood, is linked to the development of various cancers, including nasopharyngeal carcinoma, gastric cancer, Burkitt lymphoma, non-Hodgkin lymphoma (NHL), and Hodgkin lymphoma. We reviewed the literature published since 1980 regarding an association between antibodies against EBV proteins and the risk of EBV-associated malignancies. Immunoglobulin A antibody levels that are elevated before diagnosis have consistently been associated with the risk of nasopharyngeal carcinoma, and patients with Hodgkin lymphoma have significantly higher immunoglobulin G antibody levels than disease-free controls. However, the link between the immune response to EBV and other EBV-associated malignancies was less clear. Although evidence of an association between the risk of Burkitt lymphoma and immunoglobulin G antibodies was consistent for available studies, the sample sizes were limited. Evidence for a link between antibodies against EBV and risk of either gastric cancer or NHL was inconsistent. Future investigations should account for tumor EBV status because only 7%–10% of gastric tumors and select NHL subtypes are related to EBV infection. Comparing differences in the associations between the humoral immune response to EBV and disease risk across cancers may help elucidate how this ubiquitous virus contributes to distinct tumors globally.

Burkitt lymphoma; EBV antibodies; EBV immune response; EBV serology; gastric carcinoma; Hodgkin lymphoma; nasopharyngeal carcinoma; non-Hodgkin lymphoma

Abbreviations: BL, Burkitt lymphoma; CI, confidence interval; EA, early antigen; EBNA, EBV nuclear antigen; EBV, Epstein-Barr virus; GC, gastric cancer; HL, Hodgkin lymphoma; IgA, immunoglobulin A; IgG, immunoglobulin G; NHL, non-Hodgkin lymphoma; NPC, nasopharyngeal carcinoma; VCA, viral capsid antigen

Epstein-Barr virus (EBV), a human herpes virus primarily transferred by oral secretions, persists as a latent infection in human memory B-cells (1–5). Although the average age of childhood infection varies according to geographic region, more than 90% of adults globally are estimated to be infected with EBV (6). In developed countries such as the United States, more than 80% of persons who are tested for EBV are found to be positive by the age of 19 years (7). Malignant transformation of B-cells or epithelial cells infected with EBV can result in the development of various EBV-associated cancers in both children and adults.

Although EBV is a ubiquitous pathogen, infection only manifests as symptomatic disease in a subset of individuals who harbor the virus. The important question of whether markers of the human immune response to the virus, including antibody patterns, can identify which individuals are at increased risk of disease remains inadequately answered for most EBV-associated malignancies. Evidence does exist to indicate that altered anti-EBV antibody levels, particularly levels of immunoglobulin A (IgA) antibodies targeted against lytic and latent proteins expressed at mucosal surfaces, are predictors of the development of nasopharyngeal carcinoma (NPC) (8–11). Work is ongoing in high-incidence regions to incorporate these anti-EBV antibodies as potential screening biomarkers to aid in the detection and diagnosis of NPC (12, 13).

However, the number of proteins investigated to date represents only a small percentage (~5%) of the nearly 100 open
reading frames in the EBV genome that could be informative, and this limited number of antigens has only been evaluated as a discriminatory tool to identify individuals who may go on to develop NPC but no other EBV-associated malignancy. Furthermore, characterizing patterns of the humoral immune response that precede disease development may shed light on the biological degree of similarity across different EBV-associated tumors, and abnormal antibody responses against the virus may reflect systemic immunodeficiency in certain cancer patients. To begin to address these issues, we conducted a review of the literature that included research studies investigating the epidemiologic association between antibodies against EBV and the risk of developing any of the following EBV-associated malignancies: NPC, gastric cancer (GC), Burkitt lymphoma (BL), non-Hodgkin lymphoma (NHL), and Hodgkin lymphoma (HL).

METHODS

For NPC, we considered the evidence of an association between EBV serological markers (i.e., anti-EBV antibodies) measured before disease onset and the development of cancer to be strong. Therefore, an exhaustive literature review was not conducted, but key prospective studies were highlighted.

In contrast, the evidence for the other 4 EBV-associated malignancies was not considered conclusive, so we conducted an exhaustive literature review using combinations of the following search terms for each malignancy: “cancer x” and “EBV serology or EBV antibodies or EBV immune response.” This keyword search was performed in PUBMED and was restricted to English-language articles published after 1980.

Results generated through this keyword search are reported with an emphasis on 2 characteristics: 1) EBV proteins targeted and 2) antibody type. The EBV proteins targeted included structural proteins expressed during the lytic cycle, such as viral capsid antigen (VCA). Nonstructural proteins were also targeted, including both those crucial for viral genome maintenance inside latently infected cells, such as EBV-nuclear antigen (EBNA), and those expressed during the intracellular switch from latent phase to lytic viral replication, such as early antigen (EA) and ZEBRA.

The antibody types highlighted include immunoglobulin G (IgG) and IgA. IgG antibodies are the most abundant form of antibodies produced by plasma cells in response to exposure to infectious agents and typically reflect cumulative exposure to EBV (14). IgA antibodies are produced at mucosal surfaces and are thought to be markers of more recent exposure in these compartments, such as the oral or nasopharyngeal epithelium (14).

NASOPHARYNGEAL CARCINOMA

EBV is a necessary cause of undifferentiated NPC, with tumors displaying an EBV latency program characterized by the expression of EBNA1 and at least 1 of the latent membrane proteins (latent membrane proteins 1–2) (15–21). In multiple case-control studies conducted in previous decades, associations between the presence of elevated anti-EBV antibody titers and NPC were found (22–27). More recent evidence from prospective studies in populations at high risk of undifferentiated NPC has confirmed these earlier observations and demonstrated that high antibody titers, particularly IgA antibodies directed against EBV structural proteins, precede the development of NPC. Taiwanese men who tested positive for VCA IgA antibodies had a higher risk of developing NPC than did men who tested negative for VCA IgA (hazard ratio = 22.0, 95% confidence interval (CI): 7.3, 66.9), even 5 or more years after antibodies were measured (hazard ratio = 13.9, 95% CI: 3.1, 61.7) (9).

In a longitudinal study from China, Ji et al. (10) described a characteristic window approximately 3 years before NPC diagnosis during which VCA IgA antibodies are elevated in the majority of individuals who develop the disease. Additional recent work in a high-risk region of China has provided a strong addition to the connection between pre-diagnostic EBV serological markers and NPC by describing a dose-dependent relationship, linking higher VCA IgA titers to elevated risk of NPC, and reporting higher disease risk in individuals with increasing versus stable VCA IgA titers over time (hazard ratio = 12.4, 95% CI: 5.4, 28.5) (8).

Furthermore, in a prospective study of members of high-risk Taiwanese multiplex families, which are defined as families with at least 2 first- or second-degree relatives who have been diagnosed with NPC, Yu et al. (11) observed elevated levels of both VCA IgA and IgA antibodies against the non-structural EBNA1 protein before NPC diagnosis. Individuals who were positive for EBNA1 IgA at baseline experienced higher rates of NPC over follow-up (median, 6.5 years) than did persons who tested negative for EBNA1 IgA (relative risk = 6.6, 95% CI: 1.5, 61).

GASTRIC CARCINOMA

GC represents another EBV-associated malignancy that arises in epithelial cells, although unlike NPC, evidence of a link between antibodies against EBV and GC risk is inconsistent (Table 1, Web Table 1 (available at http://aje.oxfordjournals.org/)). One important contrast between GC and NPC is that only a minority of GC cases are associated with EBV infection, with approximately 7%–10% of tumors classified as EBV-related (28).

We identified 3 prospective cohort studies (29–31) and 1 case-control study (32) that evaluated the relationship between GC and EBV serological markers, with sample sizes ranging from 54 to 185 GC cases. Two of these 4 studies did not make the important distinction between EBV-positive and EBV-negative GC but rather included a single case group. No statistically significant increases in GC risk were seen in subjects with elevated antibody titers against either structural (VCA) or nonstructural (EBNA1, EA) EBV proteins in these 2 prospective studies (29, 30). Investigators in the Chinese study (n = 185 cases) instead reported an inverse association between elevated VCA IgG antibody titers measured before diagnosis and GC risk, an association that was more pronounced after analyses were restricted to cases who were diagnosed at least 2 years after blood draw (odds ratio = 0.60, 95% CI: 0.36, 0.99) (30). This inverse association between higher VCA IgG titer and lower GC risk was 1) more pronounced for cardia GC cases than for noncardia
EBV Antibodies and EBV-Related Cancer Risk

Table 1. Gastric Carcinoma Studies That Were Reviewed, 1995–2009

<table>
<thead>
<tr>
<th>First Author, Year (Reference No.)</th>
<th>EBV Protein</th>
<th>Antibody Type</th>
<th>Study Design</th>
<th>No. of Controls</th>
<th>No. of Cases</th>
<th>IgG Association†</th>
<th>IgA Association†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Levine, 1995 (31)</td>
<td>VCA EBNA EA</td>
<td>IgG, IgA, IgG</td>
<td>Prospective</td>
<td>54</td>
<td>54</td>
<td>Positive</td>
<td>Null</td>
</tr>
<tr>
<td>Shinkura, 2000 (32)</td>
<td>VCA EBNA EA</td>
<td>IgG, IgA, IgG</td>
<td>Case-control</td>
<td>73</td>
<td>123</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>Koshiol, 2007 (30)</td>
<td>VCA EBNA EA</td>
<td>IgG, IgG, IgA</td>
<td>Prospective</td>
<td>200</td>
<td>185</td>
<td>Inverse</td>
<td>Null</td>
</tr>
<tr>
<td>Kim, 2009 (29)</td>
<td>VCA EBNA EA</td>
<td>IgG, IgA, IgG</td>
<td>Prospective</td>
<td>200</td>
<td>100</td>
<td>Null</td>
<td>Null</td>
</tr>
</tbody>
</table>

Abbreviations: EA, early antigen; EBNA, EBV nuclear antigen; EBV, Epstein-Barr virus; IgA, immunoglobulin A; IgG, immunoglobulin G; VCA, viral capsid antigen.

a A positive association indicates that the study found a significantly higher risk of cancer associated with elevated antibody levels; a null association indicates that the study found no significant association between cancer risk and antibody levels; and an inverse association indicates that the study found a significantly lower risk of cancer associated with elevated antibody levels.

cases and 2) in the opposite direction of that observed between EBV serological markers and risk for all other EBV-associated malignancies.

The other 2 studies did make the important distinction between EBV-positive and EBV-negative GC tumors (31, 32). In a prospective cohort of Japanese men in whom anti-EBV antibodies were measured in serum collected an average of 13.7 years (range, 2.7–21.3 years) before GC diagnosis, EBV-positive cases (n = 14) had higher geometric mean titters of IgG antibodies to both structural (VCA) and nonstructural (EBNA1) proteins than did EBV-negative cases (n = 40) and controls (n = 54) (31). A second Japanese study in which antibodies were measured at the time of GC diagnosis also found higher VCA IgG geometric mean titers in EBV-positive cases (n = 64) than in EBV-negative cases (n = 59) and controls (n = 73) (32). Additionally, seropositivity rates for VCA IgA and EA IgG in that case-control study were significantly higher in EBV-positive cases than in EBV-negative cases (odds ratio ≈7 for VCA IgA and odds ratio ≈20 for EA IgG).

Although both studies reported increased risk of EBV-positive GC in subjects with higher VCA antibody titers, the sample size in the earlier study was limited (e.g., fewer than 20 EBV-positive cases). In addition, the data did not elucidate the importance of the timing of antibody measurement, with one study collecting blood at the time of GC diagnosis and the other measuring antibodies in blood collected 2.7–21.3 years before diagnosis. Of note, results from both of these studies contrasted with the inverse association observed between VCA titers and GC risk in the larger prospective study (n = 185 cases) that did not consider EBV-positive and EBV-negative GC tumors separately (30).

Unlike other EBV-associated malignancies, GC has potential precursor lesions that have been identified and can be evaluated in relation to EBV serological markers. In a longitudinal Chinese study of gastric lesions, persons with elevated baseline VCA IgG and EBNA IgG titers had a higher likelihood of progressing to more severe gastric dysplasia 2 years later, particularly subjects with baseline diagnoses of intestinal metaplasia (33). Ascertainment of varying gastric neoplastic states of disease could allow for larger statistically powered studies to evaluate the link between EBV serological markers and gastric carcinogenesis.

BURKITT LYMPHOMA

BL is a lymphoid malignancy that in its endemic form is nearly uniformly associated with EBV (34) (Table 2, Web Table 2) and predominantly affects children in East Africa, where a link between the presence of anti-EBV antibodies and BL risk was established through a large (n = 42,000) prospective study conducted among Ugandan children who were 0–8 years of age in the 1970s (35, 36). The original report included 14 incident cases ascertained over 5 years (35). After an additional 3 years of follow-up, 16 cases had developed (36), and titers of IgG antibodies against the structural protein VCA, although uniformly present in these children as a marker of EBV seropositivity, were significantly higher in those who developed BL. Of note, this was the only prospective study to date in which the relationship between prediagnostic EBV serological markers and risk of BL was investigated, and no studies to date have evaluated IgA antibodies.

Subsequently, 1 community-based and 2 hospital-based case-control studies that evaluated IgG antibodies have been conducted in African countries, with sample sizes ranging from 32 to 173 BL cases (37–39). These studies confirmed that children with higher VCA IgG titers had a higher BL risk regardless of the assay used, including the older immunofluorescence test (39), the more standardized enzyme-linked immunosorbent assay (38), or a multiplex bead assay (37). The most recent case-control study evaluated IgG antibody titers against nonstructural proteins such as EBNA1 and EA (37), but in accordance with the original studies conducted in Uganda (35, 36), titers were not significantly different between BL cases and controls.

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IgG antibody titers against the ZEBRA protein, which is involved in the switch from the latent phase to lytic EBV replication, were also evaluated in the most recent case-control study. No odds ratio estimates for seropositivity according to BL status were presented, but the study found that ZEBRA IgG titers were significantly elevated among the 32 BL cases compared with the 25 controls (37). Confirmation of this finding, as well as the lack of association of BL with non-structural proteins (i.e., EBNA1), requires additional studies that include larger case numbers, are well controlled (i.e., either prospectively conducted or utilize community rather than hospital controls), and measure IgA (not just IgG) antibodies against EBV proteins.

NON-HODGKIN LYMPHOMA

The evidence of a link between anti-EBV antibody titers and NHL risk is inconclusive (Table 3, Web Table 3). The identification of EBV genomes and expression of oncogenic EBV proteins (i.e., latent membrane protein 1) in malignant cells has been demonstrated for only select subtypes of NHL in immunocompetent persons, including T-cell and central nervous system lymphomas (40–42). In contrast, the fraction of lymphoproliferative tumors attributable to EBV is higher for cancers that occur in patients with acquired immunodeficiency syndrome (43, 44). Nearly 100% of central nervous system lymphomas and substantive percentages (as high as 65%–75%) of certain systemic lymphomas are related to EBV infection in these immunosuppressed patients (45–47). Persistent B-cell stimulation by long-term EBV infection offers a possible mechanism via which the virus could indirectly alter the risk of B-cell lymphomas in addition to the potential directly oncogenic effects of EBV transcripts (48–51).

We identified 8 studies, ranging in sample size from 11 to 491 NHL cases, that were conducted among immunocompetent individuals without stratification by tumor EBV status. Two of the prospective cohort studies investigated NHL risk in relation to IgA antibodies but found no associations (52, 53). Most studies evaluated the IgG responses to VCA and EA, and 4 of the 8 studies evaluated IgG response to EBNA in relation to NHL risk. One case-control study (54), as well as 3 of the 6 prospective studies reviewed here, reported no association between NHL risk and altered IgG antibody levels against any of these EBV proteins (52, 55, 56).

Results across the case-control study and 3 prospective studies with significant findings were conflicting (53, 57, 58, 59). An analysis utilizing serum from a 4-cohort collaboration (n = 104 cases) showed a higher risk of NHL associated with elevated VCA IgG titers (hazard ratio = 3.3, 95% CI: 3.3, 95% CI: 1.2, 8.8) and a lower risk of NHL associated with lower EBNA IgG titers (hazard ratio = 0.2, 95% CI: 0.04, 0.9) (53). However, this risk pattern was limited to cases diagnosed within 5 years of the blood draw (n = 56), with no association observed between IgG titers and NHL diagnosed at least 5 years after the blood draw (n = 48). In contrast, a slightly smaller prospective study (n = 73 cases) found that individuals who were seropositive for EA IgG were at a higher risk of developing NHL after over a decade (≈12 years) of study follow-up but observed no association with VCA IgG seropositivity (57), results that were supported by similar case-control findings of elevated IgG titers against EA but not VCA in blood drawn at the time of NHL diagnosis (58). Finally, the largest prospective cohort to date (n = 491 cases) reported no increase in B-cell lymphoma risk for persons with elevated IgG titers against either VCA or EA; rather, persons who were seropositive for EBNA1 IgG were observed to instead have a lower risk of NHL, irrespective of the timing between blood draw and diagnosis (59).

Two of the studies reviewed here, each nested within a large US cohort, reported results by NHL subtype, and both observed suggestions of a higher risk associated with elevated anti-EBV antibodies that was specific to chronic lymphocytic leukemia, small lymphocytic lymphoma, and prolymphocytic lymphoma subtypes (55, 59). Investigators from the Physicians’ and Nurses’ Health Studies (n = 79 chronic lymphocytic leukemia/small lymphocytic lymphoma cases) reported a higher risk associated with elevated EBNA2 IgG titers (relative risk = 1.74, 95% CI: 0.99, 3.05) (55). In the Women’s Health Initiative Study (n = 142 chronic lymphocytic leukemia/small lymphocytic lymphoma/prolymphocytic lymphoma

<table>
<thead>
<tr>
<th>First Author, Year (Reference No.)</th>
<th>EBV Protein</th>
<th>Antibody Type</th>
<th>Study Design</th>
<th>No. of Controls</th>
<th>No. of Cases</th>
<th>IgG Association*</th>
<th>IgA Association*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Geser, 1982 (36)</td>
<td>VCA</td>
<td>IgG</td>
<td>Prospective</td>
<td>80</td>
<td>16</td>
<td>Positive</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>EBNA</td>
<td>IgG</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>EA</td>
<td>IgG</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carpenter, 2008 (38)</td>
<td>VCA</td>
<td>IgG</td>
<td>Case-control</td>
<td>102b</td>
<td>173</td>
<td>Positive</td>
<td>NA</td>
</tr>
<tr>
<td>Mutalima, 2008 (39)</td>
<td>VCA</td>
<td>IgG</td>
<td>Case-control</td>
<td>89b</td>
<td>128</td>
<td>Positive</td>
<td>NA</td>
</tr>
<tr>
<td>Asito, 2010 (37)</td>
<td>VCA</td>
<td>IgG</td>
<td>Case-control</td>
<td>25</td>
<td>32</td>
<td>Positive</td>
<td>NA</td>
</tr>
</tbody>
</table>

Abbreviations: EA, early antigen; EBNA, EBV nuclear antigen; EBV, Epstein-Barr virus; IgA, immunoglobulin A; IgG, immunoglobulin G; NA, not applicable; VCA, viral capsid antigen.

* A positive association indicates that the study found a significantly higher risk of cancer associated with elevated antibody levels; NA indicates that the study did not evaluate the association.

b Hospital controls.
cases), seropositivity for EA IgG was significantly associated with elevated disease risk (relative risk = 1.8, 95% CI: 1.2, 2.6), although the risk was more pronounced for cases diagnosed within 4 years of blood draw (59).

Additionally, work has been done to characterize patterns of multiple IgG anti-EBV antibodies simultaneously in lymphoma patients through immunoblot analysis (60). The immunoblot assay classifies the overall IgG response for a person as either 1) normal, with reactivity to a limited number of peptides (e.g., EBNA1, VCAp18, VCAp40, ZEBRA) or 2) abnormal, with recognition of a higher diversity of EBV peptides. This work has provided evidence that the abnormal humoral immune response pattern is more prevalent among persons with the chronic lymphocytic leukemia lymphoma subtype (odds ratio = 2.96, 95% CI: 2.22, 3.95). However, this immunoblot assay is not quantitative and does not provide a titer level for each individual peptide recognized. Future work is warranted to attempt to reproduce these patterned findings using a quantitative assay.

HODGKIN LYMPHOMA

HL is the only EBV-related malignancy other than NPC for which there is a body of evidence accumulated over time that established a strong association between altered EBV humoral immune responses and development of disease, with most work to date focused on IgG antibodies (Table 4, Web Table 4). Numerous case-control studies were conducted before 1980, many of which were summarized in an article by Evans et al. published in 1984 (61). In brief, these early studies reported significantly higher IgG titers associated with HL status, with 14 of 18 studies evaluating VCA IgG and 6 of 8 studies evaluating EA IgG reporting higher prevalence of elevated titers in HL cases than in controls. Rather than reiterate what has already been summarized, below we focus on the 4 studies published on this topic since 1980, which included sample sizes ranging from 43 to 189 HL cases.

Approximately 30%–40% of HL tumors in immunocompetent persons are estimated to be related to EBV (1, 62), although this proportion can be 80% or higher in persons who are infected with human immunodeficiency virus (45, 63, 64). The only study reviewed here that stratified HL tumors by EBV status reported that higher VCA, EA, and EBNA IgG antibody levels were specifically associated with elevated risk for EBV-positive HL (n = 40 cases) but not EBV-negative HL (n = 88 cases) (65).

The other 3 studies (61, 66, 67) did not make a distinction between EBV-positive and EBV-negative HL tumors. However, unlike for the risks of GC and NHL, associations between EBV serological markers and HL risk were consistent even when considering one HL case group. One potential explanation is that a much higher percentage of HL tumors (≈50%) are EBV related compared with GC or NHL tumors (<10%). The VCA IgG and IgA response, as well as IgG responses to EBNA and EA, were significantly elevated in HL patients compared with controls when measured both after HL diagnosis (61) and before the development of HL (66). The prospective study reported that the association between
IgG antibody titers and HL risk was actually stronger after a longer latency period (66). For example, persons who developed HL were more than twice as likely to have elevated VCA IgG titers (relative risk = 2.6, 95% CI: 1.1, 6.1), but those who were diagnosed with HL at least 3 years after study enrollment were nearly 5 times as likely to have elevated VCA IgG titers (relative risk = 4.9, 95% CI: 1.5, 16.3). Interestingly, this stronger association between anti-VCA antibodies measured years before HL diagnosis stands in contrast to the association of these same antibodies with short-term but not longer-term NHL risk in the very same persons (53, 66).

Two studies reviewed here investigated not only single markers of the humoral immune response to EBV but also aberrant serological patterns that could reflect immune dysfunction (65, 67). The EBNA2 protein is involved in the transformation of EBV-infected lymphocytes (1, 3). High anti-EBNA2 antibody titers are therefore observed after primary infection, but anti-EBNA2 antibody titers should decrease after resolution of acute infection in parallel with elevation of antibodies against the less immunogenic EBNA1 protein necessary for maintenance of latent infection (67–69). The presence of an altered EBNA IgG ratio in which EBNA2 antibodies do not decline over the course of infection (EBNA1: EBNA2 ratio ≤1.0) is a phenomenon observed in patients with immunological disorders (68). In the 2 studies reviewed here, this altered EBV immune response was associated with an increased risk of HL specific to EBV-positive tumors (65) and for HL diagnosed in persons with no history of infectious mononucleosis (67).

### DISCUSSION

The strength of evidence in the current literature for an association between anti-EBV antibody titers and risk of EBV-associated malignancies differs according to cancer diagnosis. Having elevated levels of anti-EBV IgA antibodies is related to the development of incident NPC. Although the examination of IgA antibodies is limited in relation to HL, the evidence for a link between anti-EBV IgG antibody titers and HL risk is strong. The evidence presented for elevated VCA IgG response and BL status was consistent; however, sample sizes were limited in 2 of the 4 reviewed studies, and the other 2 utilized hospital-based controls, a potentially biased comparison group. Data for GC are inconsistent, with some evidence observed for a link between the anti-VCA response and EBV-positive gastric tumors. Finally, for NHL, half of the studies reviewed reported null results, although some prospective data indicate that the presence of altered anti-EBV antibodies may be important for certain subtypes of NHL.

Although evidence for an association between elevated IgA antibody titers and NPC risk is strong, IgA antibodies against only 3 EBV proteins (EBNA1, VCA, and EA) have been consistently investigated in NPC studies to date (8–11). We observed that this same set of 3 proteins represented the universe of investigated EBV targets in the available literature for the other EBV-associated malignancies. This comprises only a small fraction of the approximately 100 open reading frames for EBV, meaning that little comprehensive investigation of EBV serological patterns has been conducted. The technology to explore antibody patterns and investigate responses to multiple peptides simultaneously is available (60, 70, 71). For example, one of the BL studies reviewed here utilized multiplex bead technology to quantitate the response to multiple peptides in a single assay (37). Expanding on the number of antigens evaluated could deepen our understanding of the role of the immune response to EBV in the development of these cancers. If it is consistently demonstrated that IgA antibodies are preferentially important for NPC, whereas IgG antibodies are preferentially important for HL, this might reinforce the notion that control of lytic replication at mucosal epithelial surfaces is an important predictor for NPC, whereas the expansion of the EBV-infected B-cell pool is crucial for HL.

Results from lymphoma work reviewed here support the importance of investigating not just individual antibody titers but also patterns of the humoral immune response to EBV (60, 67). Elevations of specific antibodies years before disease onset may support a direct oncogenic role for EBV in certain cancers, but altered antibody patterns close to the time of diagnosis could reflect a higher prevalence of impaired immunity in persons who develop these malignancies. Both alternatives deserve further evaluation.
Beyond etiological insights, the link between anti-EBV antibodies and disease risk may ultimately translate into biomarkers that can identify persons who are more likely to develop disease. Although it is premature to consider using EBV serological markers for population-level cancer prevention efforts, an endeavor that may never be justified for certain EBV-associated cancers with low incidence or small percentages of EBV-related disease, in at least one case what we have learned about etiologies of these cancers can be applied toward prevention efforts. Measurement of IgA antibody levels has been used in a large, general-population NPC screening demonstration project conducted in a high-risk region of China (72–75). Studies conducted in this region during the 1980s found rates of NPC among those that screened positive for VCA IgA that were approximately 40 times higher than those observed in the cohort at large. Unfortunately, these early efforts were not carefully controlled and do not permit accurate quantification of the potential impact of EBV serology–based screening on detection or mortality rates of NPC. Since that time, enzyme-linked immunosorbent assay methods that are cheaper and easier to standardize than the immunofluorescence tests used in the 1980s have become available. Current work in this region of China is ongoing to evaluate the utility of IgA antibodies against VCA and EBNA1 as screening biomarkers, which will in turn allow us to evaluate whether implementation of EBV serology–based screening can decrease the NPC mortality rate (12, 13).

Our findings suggest that the relationship between the antibody response to EBV and the risk of developing EBV-associated tumors is not well characterized for at least 3 of the 5 cancers reviewed. The results from studies of the different types of cancer often conflict, evaluate antibodies against a limited number of EBV proteins, or fail to investigate multiple antibody types (e.g., IgG and IgA), thereby limiting our ability to draw definitive conclusions about the role of the humoral immune response to EBV in predicting disease risk. However, the available evidence does point to an association between EBV serological patterns and risk for certain cancers, and the availability of reproducible and affordable technology to measure antibody levels presents us with an opportunity to elucidate the role of the humoral immune response to EBV in cancer etiology and prevention moving forward.

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