Check for updates

A prospective study of Epstein-Barr virus antibodies and risk of non-Hodgkin lymphoma

Kimberly A. Bertrand,[1,2] Brenda M. Birmann,[2] Ellen T. Chang,[3,4] Donna Spiegelman,[1,5] Jon C. Aster,[6] Shumin M. Zhang,[7] and Francine Laden[1,2,8]

[1]Department of Epidemiology, Harvard School of Public Health, Boston, MA; [2]Channing Laboratory, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA; [3]Cancer Prevention Institute of California, Fremont, CA; [4]Division of Epidemiology, Department of Health Research and Policy, Stanford University School of Medicine, Stanford, CA; [5]Department of Biostatistics, Harvard School of Public Health, Boston, MA; [6]Department of Pathology, Brigham and Women's Hospital and Harvard Medical School, Boston, MA; [7]Division of Preventive Medicine, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA; and [8]Department of Environmental Health, Harvard School of Public Health, Boston, MA

Severe immunosuppression is an established risk factor for non-Hodgkin lymphoma (NHL), but an association with subclinical immune dysfunction is unclear. We conducted a case-control study nested in the Physicians' Health Study and the Nurses' Health Study cohorts to determine whether patterns of antibody response to Epstein-Barr virus (EBV) were associated with NHL risk. We measured antibody titers against viral capsid antigen, early antigen, and Epstein-Barr nuclear antigen (EBNA-1 and EBNA-2) in blood samples collected before diagnosis from 340 cases and 662 matched controls. Using conditional logistic regression, we estimated rate ratios (RRs) and 95% confidence intervals (CIs) for elevated versus normal titers and the ratio of anti–EBNA-1 to anti–EBNA-2 titers (≤ 1.0 vs > 1.0). We found no association between EBV serostatus, elevated titers, or an EBNA-1/EBNA-2 ratio ≤ 1.0 and NHL risk overall. For chronic lymphocytic leukemia/small lymphocytic lymphoma, suggestive associations were noted for elevated anti–EBNA-2 (RR, 1.74; 95% CI, 0.99-3.05), anti–viral capsid antigen (RR, 1.58; 95% CI, 0.79-3.14), and EBNA-1/EBNA-2 ratio ≤ 1.0 (RR, 1.52; 95% CI, 0.91-2.55). There was no evidence of heterogeneity by subtype. Overall, we found no evidence that EBV antibody profile predicts NHL risk in immunocompetent persons, with the possible exception of chronic lymphocytic leukemia/small lymphocytic lymphoma. (*Blood*. 2010;116(18):3547-3553)

Introduction

In the United States, 81 470 new cases of non-Hodgkin lymphoma (NHL) were expected to be diagnosed in 2009.[1] The most established risk factor for NHL is immune deficiency, including inherited and acquired immunosuppression. Certain infections have also been linked to specific NHL subtypes. Most NHL diagnoses, however, occur in apparently immunocompetent persons with no known risk factors.

Epstein-Barr virus (EBV) is a ubiquitous herpesvirus that infects > 90% of the human population and establishes persistent (lifelong) latent infection in the host.[2] The serologic responses to EBV infection have been well characterized.[3] Primary infection is usually asymptomatic, although a subset of persons in whom primary infection is delayed until adolescence or young adulthood develop infectious mononucleosis (IM).[4] On primary infection with EBV, antibodies to viral antigens expressed during lytic replication, viral capsid antigen (VCA) and early antigen (EA), appear first.[3] Antibodies to Epstein-Barr nuclear antigen-2 (EBNA-2) are the first to emerge against a latent cycle antigen. These reach peak levels and then decline over subsequent months to persistent low or nondetectable levels.[3] Anti–EBNA-1 is first apparent after anti–EBNA-2 and gradually increases in titer, ultimately reaching a level that persists indefinitely.[3] EBV persists as a latent infection in memory B lymphocytes,[5] although reactivation of the virus commonly occurs, usually without symptoms.[6]

Of relevance to the present study, immunocompromised persons and those with chronic IM display an altered EBV serologic profile characterized in part by persistently elevated anti–EBNA-2 titers and reduced anti–EBNA-1 titers.[3,7] In addition, compared with healthy controls, patients with chronic EBV, NHL, Hodgkin lymphoma, and nasopharyngeal carcinoma have higher titers of immunoglobulin G (IgG) antibodies to VCA and EA.[7] Patients with severe clinical immune deficiencies also show high anti-VCA and anti-EA titers, a pattern consistent with compromised cellular immune control of EBV.[8]

Although EBV infection is benign in most persons, EBV is a known carcinogen[9] and has been specifically linked to the etiology of nasopharyngeal carcinoma, Hodgkin lymphoma, endemic Burkitt lymphoma, nasal T/natural killer–cell lymphomas, and several rare AIDS- and associated posttransplantation B-cell lymphomas.[10] There is strong evidence that EBV infection in combination with severe immune dysfunction is associated with increased risk of NHL.[11] A causative role of EBV in more common NHL subtypes and in the absence of severe immune deficiency is suspected but remains unproven. An abnormal antibody response to EBV could serve as either an indicator of EBV involvement in NHL development or a nonspecific marker of underlying (ie, subclinical) immune dysfunction.

Few studies have examined the association of EBV with NHL in patients not known to be immunosuppressed.[12-17] Although these

Submitted May 3, 2010; accepted July 12, 2010. Prepublished online as *Blood* First Edition paper, July 20, 2010; DOI 10.1182/blood-2010-05-282715.

The publication costs of this article were defrayed in part by page charge payment. Therefore, and solely to indicate this fact, this article is hereby marked "advertisement" in accordance with 18 USC section 1734.

© 2010 by The American Society of Hematology

Downloaded from http://ashpublications.org/blood/article-pdf/116/18/3547/1330722/zh804410003547.pdf by guest on 21 May 2024

studies provide some evidence that NHL cases as a group may have abnormal antibody responses to EBV before disease, the specific pattern is not clear and the laboratory assays are not mutually comparable. To test the hypothesis that profile of antibody response to EBV is associated with risk of NHL, we conducted a nested case-control study that used prospective blood samples from men and women in the Physicians' Health Study (PHS) and Nurses' Health Study (NHS) cohorts. We evaluated these associations of EBV antibody profile with all NHL and with common subtypes of NHL as defined by the World Health Organization (WHO) classification of lymphomas.[18]

Methods

Study population

The present study was conducted in the PHS and the NHS cohorts. The PHS began in 1982 as a randomized trial of aspirin use and β-carotene in the primary prevention of cardiovascular disease and cancer among 22 071 US male physicians ages 40-84 years at enrollment.[19] Baseline information, including age, smoking history, weight, height, and race, was collected by self-administered questionnaire. Follow-up in the PHS is 97% complete for morbidity and mortality.[20] Between August 1982 and December 1984 (before randomization), 14 916 men provided a baseline blood sample with the use of blood collection kits sent to the participants. Plasma and whole blood specimens were received in our laboratory on chill packs within 24 hours of being drawn. On arrival, the samples were refrigerated, redivided into aliquots, and frozen at −82°C.

The NHS was established in 1976 when 121 700 female registered nurses aged 30-55 years living in 11 US states completed a self-administered questionnaire on risk factors for cancer and other diseases. Every 2 years, questionnaires have been sent to cohort members to update information on potential risk factors and to identify newly diagnosed cancers and other diseases. Vital status is ascertained through next of kin and the National Death Index; both methods identified an estimated 98% of deaths in the cohort.[21] From May 1989 to September 1990, blood samples were collected from a subcohort of 32 826 women in the NHS by overnight courier; 97% of samples arrived within 26 hours of being drawn. On arrival, samples were centrifuged and redivided into aliquots of plasma, white blood cell, and red blood cell components and stored at −130°C.

Incident NHL diagnoses, including those of chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL), in the PHS and NHS blood subcohorts were identified by follow-up questionnaires and were confirmed by review of medical records and pathology reports. We excluded men and women with a diagnosis of NHL before or within 6 months of blood collection and those with a prior diagnosis of cancer (other than nonmelanoma skin cancer). Histologic subtype was determined according to the WHO classification.[18] Specifically, diagnoses were made on the basis of morphology and immunophenotype information available in medical records and pathology reports and represented the consensus opinion of 2 hematopathologists. Immunophenotype was not required for tissue diagnoses of CLL/SLL or follicular lymphoma, which can be reliably diagnosed by morphology alone.[18] We identified 350 NHL cases (205 men and 145 women) diagnosed between 1982 and 2003 with available blood samples; of these, 79 were CLL/SLL, 66 were diffuse large B-cell lymphoma (DLBCL), and 54 were follicular lymphoma (FL). The remaining cases included 72 patients with uncommon or unspecified B-cell histology, 19 patients with T-cell lymphoma, and 50 patients who were determined to have NHL on the basis of morphology alone but lacked adequate phenotyping to assign the tumor to the B- or T-cell lineage.

For each case, 2 control subjects with no history of cancer (except nonmelanoma skin cancer) were selected at random from among the PHS and NHS participants who were of the same sex, race, and age at baseline (± 1 year), who provided a blood sample at the same time (± 1 month), and who were at risk of NHL at the same time a case occurred. Cases and controls were also matched on fasting status at blood draw.

This study was approved by the Institutional Review Board of Brigham and Women's Hospital, and all participants provided written informed consent at initial enrollment in accordance with the Declaration of Helsinki.

Laboratory analyses

Analyses were done at the Swedish Institute for Infectious Disease Control Department of Virology laboratory. Laboratory personnel were blinded to case-control status. The laboratory methods have been described in detail elsewhere.[22,23] IgG antibodies to EBV VCA and EA (diffuse and restricted) were determined by indirect immunofluorescence. For samples with detectable anti–EA-restricted and anti–EA-diffuse IgGs, the higher value was taken to be the titer against EA, as in other studies.[24] IgG antibodies against EBNA-1 and EBNA-2 were determined by enzyme-linked immunosorbent assay. Antibody titers are reported as the reciprocal of the highest of serial 2-fold dilutions to yield a positive reading on immunofluorescence. Persons were considered EBV seropositive (reflecting past infection) if the IgG antibody titer to VCA was ≥ 1:20. Ten NHL cases (6 from the PHS cohort and 4 from the NHS cohort) showed nonspecific serum reactivity to EBNA and were removed from analyses along with their matched controls. Seventeen additional control subjects (13 from the PHS cohort and 4 from the NHS cohort) also showed nonspecific serum reactivity to EBNA and were removed from analyses.

Plasma sample sets were constructed to contain matched triplets of cases and controls (with the order of samples randomized within a given set). In addition, every batch included a pair or triplet from a plasma pool used for quality control (QC). To assess the reproducibility of the titers in QC samples, we expressed the titers as the number of serial dilutions and computed the difference in dilutions between repeat samples by assay batch. We observed good reproducibility of the EBV antibody assays; intrabatch dilution differences in titers were 0 or 1 for most of the 37 batches (range, 62% for anti-EA to 92% for anti–EBNA-2). Titer measurements varied by 2 dilutions in a minority of batches; only one batch (in the NHS cohort) resulted in ≥ 3 intrabatch dilution differences between QC samples for anti–EBNA-1, anti–EBNA-2, and anti-VCA. We performed sensitivity analyses, excluding this batch.

Statistical analyses

We classified abnormal EBV antibody titers on the basis of the distribution of controls who were EBV seropositive, whom we identified on the basis of a positive titer (ie, ≥ 1:20) for anti-VCA. Specifically, "elevated" antibody titers were defined as those at the upper 15% (or nearest cutoff) of the respective distribution, which corresponded to anti-VCA ≥ 1:10 240, anti-EA ≥ 1:640, anti–EBNA-2 ≥ 1:80, and anti–EBNA-1 ≥ 1:320. We classified the ratio of titers against EBNA-1 and EBNA-2 (EBNA-1/EBNA-2 ratio) as > 1.0 versus ≤ 1.0 ("low"). The low ratio occurs primarily in persons with chronic or severe EBV infection, probably due to poor host immune control.[3]

For each EBV antibody variable, we used conditional logistic regression, stratifying on the matched case-control triplets, to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for elevated titers or a low EBNA-1/EBNA-2 ratio relative to the corresponding less extreme titers among EBV-seropositive persons. We also calculated the relative risk of NHL for EBV-seronegative persons compared with EBV-seropositive persons. Because of the nested case-control study design, the OR is an unbiased estimate of the incidence rate ratio (RR); therefore, we use the term RR. Analyses were performed for men and women separately and combined. We assessed statistical heterogeneity between men and women by likelihood ratio tests that compared models with interaction terms for joint levels of the exposure and cohort to main-effects-only models.

In separate analyses, we modeled the base 2 logarithm of the reciprocal of the dilution of the antibody titers as a continuous variable. On this scale, the regression coefficient estimates the logarithm of the RR associated with a 2-fold difference in titers; we doubled and exponentiated the coefficient to estimate the RR associated with a 4-fold difference. We excluded EBV-seronegative persons from these analyses; if titers to antibodies other than VCA were nondetectable, we calculated the base 2 logarithm of half of the minimum detectable titer. We also examined the possibly nonlinear relation

Downloaded from http://ashpublications.org/blood/article-pdf/116/18/3547/1330722/zh804410003547.pdf by guest on 21 May 2024

Table 1. Characteristics of non-Hodgkin lymphoma cases and controls in the Physicians' and Nurses' Health Studies

	All subjects		EBV-seropositive subjects only	
	Cases (n = 340), n (%)	Controls (n = 662), n (%)	Cases (n = 319), n (%)	Controls (n = 629), n (%)
Sex				
Male	199 (59)	384 (58)	183 (57)	366 (58)
Female	141 (41)	278 (42)	136 (43)	263 (42)
Mean (SD) age at diagnosis, y	66.4 (8.6)		66.5 (8.7)	
Mean (SD) age at blood draw, y	57.2 (8.1)	57.1 (8.0)	57.3 (8.1)	57.2 (8.0)
Mean (SD) height, in	67.9 (4.0)	67.9 (3.8)	67.9 (4.0)	67.9 (3.8)
Body mass index				
Normal (< 25 kg/m^2)	182 (54)	388 (59)	167 (52)	366 (58)
Overweight (25-29.9 kg/m^2)	133 (39)	221 (33)	128 (40)	210 (33)
Obese (≥ 30 kg/m^2)	25 (7)	52 (8)	24 (8)	52 (8)
Smoking				
Never smoker	159 (47)	308 (47)	148 (46)	294 (47)
Past smoker	138 (41)	281 (43)	129 (40)	265 (42)
Current smoker	43 (13)	70 (11)	42 (13)	67 (11)
Histologic subtype				
DLBCL	66 (19)		59 (19)	
CLL/SLL	79 (23)		71 (22)	
FL	54 (16)		52 (16)	
Other/unknown	141 (41)		137 (43)	

Body mass index was missing for 1 control; smoking history was missing for 3 controls.
DLBCL indicates diffuse large B-cell lymphoma; CLL/SLL, chronic lymphocytic leukemia/small lymphocytic lymphoma; and FL, follicular lymphoma.

between EBV antibody titers and the RR of NHL with restricted cubic splines.[25] Tests for nonlinearity were performed with the use of the likelihood ratio test, comparing nested models with only the linear term with the model including the linear and the cubic spline terms.

Multivariable conditional logistic regression models that included height as a continuous variable and indicator variables for smoking (never, past, current), body mass index (< 25, 25-29.9, ≥ 30 kg/m^2), and tertile of polychlorinated biphenyl concentration were used to assess possible confounding by these factors. These covariates were chosen on the basis of observed relationships with EBV antibodies or NHL and for consistency with previously published studies in this field.[26-29]

To evaluate a possible latency effect, we conducted unconditional logistic regression analyses of EBV serostatus and category of a given EBV antibody titer within strata of follow-up time dichotomized as < 5 years versus ≥ 5 years from baseline, controlling for the matching factors (ie, sex, baseline age, month and year of blood draw, race/ethnicity, and fasting status) in a multivariable model. Effect modification was assessed by the likelihood ratio test that compared models with an interaction term with a main-effects-only model. Possible effect modification by age, dichotomized at the median age at diagnosis in cases (66.7 years), was evaluated in the same manner.

The main analyses included all cases of NHL. We also performed separate analyses for the most common WHO-defined subtypes, that is, CLL/SLL, DLBCL, and FL. Other subtypes were represented by too few cases for meaningful analysis. To improve statistical efficiency, we performed unconditional logistic regression, adjusting for the matching factors in a multivariable model, in these analyses so that we could use all the controls. We used polytomous logistic regression to test for heterogeneity in effect estimates by NHL subtype.

All statistical tests were 2-sided and assumed an α error of .05. Analyses were performed with SAS Version 9 for UNIX (SAS Institute Inc).

Results

After excluding subjects with nonspecific serum reactivity to EBNA, the final study population consisted of 340 NHL cases and 662 controls. Of these, 319 cases (94%) and 629 controls (95%) were EBV seropositive. Cases and controls were similar with respect to potential NHL risk factors, with the exception that cases were more likely to be overweight than the controls (Table 1). The subgroup of EBV-seropositive cases and controls had a similar distribution of these risk factors, and of NHL histologic subtype, to the full study sample.

On the basis of the conditional logistic regression analyses, no association was observed between EBV serostatus, elevated EBV antibody titers, or an EBNA-1/EBNA-2 ratio ≤ 1.0 and risk of all NHL. In simple models that adjusted for the matching factors only, RRs ranged from 0.97 (95% CI, 0.66-1.42) for anti-EA to 1.27 (95% CI, 0.84-1.93) for anti-VCA and were thus not consistent with strong positive associations with NHL risk (Table 2). Height, body mass index, smoking history, and polychlorinated biphenyl concentration did not appear to be important confounders of the observed associations in multivariable models (data not shown).

Effect estimates for elevated antibody titers against EBNA-2 and EA and a low EBNA-1/EBNA-2 ratio were similar for men and women when computed separately by sex (all P values for interaction $> .45$). We observed a suggestive positive association of NHL risk with elevated anti–EBNA-1 among women only: the multivariable RR was 1.61 (95% CI, 0.92-2.83) for women and 0.78 (95% CI, 0.49-1.25) for men (P for interaction = .047). In contrast, elevated anti-VCA titers were associated with a nonsignificant 50% increase in NHL risk among men with no apparent risk in women (multivariable RR in men: 1.50; 95% CI, 0.80-2.81; RR in women: 0.88; 95% CI, 0.48-1.61; P for interaction = .16). Sensitivity analyses excluding the assay batch with poor reproducibility in the QC samples did not materially change the findings (data not shown).

In analyses stratified by time to diagnosis, no clear difference was observed between earlier (ie, < 5 years) and later NHL in the risk association with pattern of EBV antibody response (Table 3). In general, elevated EBV antibody titers were not associated with risk of NHL in either time period. In the early time period, there was a suggestion of a nonsignificantly increased risk of NHL associated with an EBNA-1/EBNA-2 ratio ≤ 1.0 (RR, 1.54; 95% CI, 0.85-2.79), whereas no association was observed in the later time period (RR, 0.95; 95% CI, 0.68-1.34). There was no evidence

Downloaded from http://ashpublications.org/blood/article-pdf/116/18/3547/1330722/zh804410003547.pdf by guest on 21 May 2024

Table 2. Association of EBV serostatus and category of EBV antibody titer with risk of all NHL

Exposure	Cases, n	Controls, n	Simple RR (95% CI)*
EBV serostatus			
Positive	319	629	Ref
Negative	21	33	1.22 (0.71-2.12)
Anti–EBNA-1, titer†			
< 320	252	505	Ref
≥ 320	67	124	1.07 (0.76-1.52)
Anti–EBNA-2, titer†			
< 80	256	512	Ref
≥ 80	63	117	1.06 (0.74-1.52)
Anti-VCA, titer†			
< 10 240	275	553	Ref
≥ 10 240	44	76	1.27 (0.84-1.93)
Anti-EA, titer†			
< 640	268	525	Ref
≥ 640	51	104	0.97 (0.66-1.42)
EBNA-1/EBNA-2 ratio†			
> 1.0	210	424	Ref
≤ 1.0	109	205	1.07 (0.79-1.44)

EBV indicates Epstein-Barr virus; NHL, non-Hodgkin lymphoma; EBNA, Epstein-Barr nuclear antigen; VCA, viral capsid antigen; EA, early antigen; RR, rate ratio; and CI, confidence interval.

*Conditional logistic regression models adjusting for the matching factors (sex/study, age, race, month and year of blood draw, fasting status).

†Among EBV-seropositive subjects only.

of statistical interaction by time period for any EBV antibody variable considered (all P values for interaction > .20). EBV seronegativity was not associated with risk of NHL diagnosed ≥ 5 years after blood draw. The apparently strong and statistically significant increase in risk of an earlier NHL diagnosis in EBV-seronegative persons was based on very small numbers and warrants cautious interpretation. Of note, there was no evidence of statistical interaction of EBV serostatus with follow-up time (P = .38; Table 3). We also found no evidence of effect modification by age at diagnosis (data not shown).

In analyses to explore heterogeneity in the associations of EBV antibody variables with NHL risk by major histologic subtypes (ie, CLL/SLL, DLBCL, and FL), we observed suggestive increased risks of CLL/SLL (RR, 2.09; 95% CI, 0.90-4.86) and DLBCL (RR, 2.38; 95% CI, 0.98-5.76) among EBV-seronegative persons compared with EBV-seropositive persons, although those results were based on small numbers of EBV-seronegative cases (Table 4). Risk of DLBCL did not appear to be associated with category of any EBV antibody titer. For CLL/SLL, suggestive associations of risk with several EBV antibody variables were noted, including elevated anti–EBNA-2 (RR, 1.74; 95% CI, 0.99-3.05), elevated anti-VCA (RR, 1.58; 95% CI, 0.79-3.14), and low EBNA-1/EBNA-2 ratio (RR, 1.52; 95% CI, 0.91-2.55). Elevated anti–EBNA-1 was the only antibody category to show a suggestive association with FL risk (RR, 1.80; 95% CI, 0.95-3.44). There was no evidence of statistical heterogeneity in effects by subtype, however (all P values for heterogeneity > .05). Statistical power was limited for the subtype-specific analyses.

When examined separately by sex, the base 2 logarithm of the reciprocal of the titer was not associated with NHL risk in men or women for any EBV antibody. In analyses with men and women combined, the multivariable RRs of all NHL associated with a 4-fold difference in antibody titers ranged from 1.00 (95% CI, 0.88-1.14) for anti–EBNA-1 to 1.09 (95% CI, 0.91-1.31) for anti-VCA and thus did not suggest a strong positive association for increasing titer of any antibody. Evaluation of restricted cubic splines did not suggest any nonlinearity in the associations of EBV antibody titers with NHL risk (data not shown). Consistent with the observation that elevated anti–EBNA-2 titers were positively associated with risk of CLL/SLL when the dichotomous (elevated vs not elevated) variable was modeled, a 4-fold increase in anti–EBNA-2 titer was associated with a significant 38% increase in risk of CLL/SLL (RR, 1.38; 95% CI, 1.04-1.83; P for trend = .03). A comparable increase in anti–EBNA-2 titer was not associated with risk of DLBCL or FL (RR_{DLBCL}, 0.95; 95% CI, 0.70-1.29; RR_{FL}, 0.83; 95% CI, 0.60-1.15). The test for heterogeneity by subtype in the association of NHL risk with anti–EBNA-2

Table 3. Association of EBV serostatus and category of EBV antibody titer with risk of all NHL, stratified by time to diagnosis

	Follow-up < 5 y			Follow-up ≥ 5 y			
Exposure	Cases, n	Controls, n	RR* (95% CI)	Cases, n	Controls, n	RR* (95% CI)	P for interaction
EBV serostatus							.38
Positive	71	156	Ref	248	473	Ref	
Negative	11	5	4.39 (1.37-14.09)	10	28	0.63 (0.30-1.34)	
Anti–EBNA-1, titer†							.22
< 320	62	130	Ref	190	375	Ref	
≥ 320	9	26	0.76 (0.32-1.79)	58	98	1.16 (0.80-1.69)	
Anti–EBNA-2, titer†							.33
< 80	60	129	Ref	196	383	Ref	
≥ 80	11	27	0.87 (0.38-2.00)	52	90	1.11 (0.76-1.64)	
Anti-VCA, titer†							.94
< 10 240	58	135	Ref	217	418	Ref	
≥ 10 240	13	21	1.36 (0.62-2.99)	31	55	1.10 (0.68-1.78)	
Anti-EA, titer†							.88
< 640	56	125	Ref	212	400	Ref	
≥ 640	15	31	1.02 (0.49-2.12)	36	73	0.91 (0.59-1.42)	
EBNA-1/EBNA-2 ratio†							.62
> 1.0	37	98	Ref	173	326	Ref	
≥ 1.0	34	58	1.54 (0.85-2.79)	75	147	0.9 (0.68-1.34)	

EBV indicates Epstein-Barr virus; NHL, non-Hodgkin lymphoma; EBNA, Epstein-Barr nuclear antigen; VCA, viral capsid antigen; EA, early antigen; RR: rate ratio; and CI, confidence interval.

*Unconditional logistic regression adjusting for the matching factors (sex/study, age, race, month and year of blood draw, fasting status).

†Among EBV-seropositive subjects only.

Downloaded from http://ashpublications.org/blood/article-pdf/116/18/3547/1330722/zh804410003547.pdf by guest on 21 May 2024

Table 4. Heterogeneity of the association of EBV serostatus and category of EBV antibody titer with risk of NHL, by major histologic subtype

		CLL/SLL		DLBCL		FL		
Exposure	**Controls, n**	**Cases, n**	**RR* (95% CI)**	**Cases, n**	**RR* (95% CI)**	**Cases, n**	**RR* (95% CI)**	***P* for heterogeneity†**
EBV serostatus								.35
Positive	629	71	Ref	59	Ref	52	Ref	
Negative	33	8	2.09 (0.90-4.86)	7	2.38 (0.98-5.76)	2	0.73 (0.17-3.19)	
Anti–EBNA-1, titer‡								.07
< 320	505	59	Ref	51	Ref	37	Ref	
≥ 320	124	12	0.78 (0.40-1.50)	8	0.67 (0.31-1.48)	15	1.80 (0.95-3.44)	
Anti–EBNA-2, titer‡								.08
< 80	512	50	Ref	46	Ref	46	Ref	
≥ 80	117	21	1.74 (0.99-3.05)	13	1.15 (0.59-2.24)	6	0.55 (0.23-1.33)	
Anti-VCA‡								.56
< 10 240	553	59	Ref	52	Ref	45	Ref	
≥ 10 240	76	12	1.58 (0.79-3.14)	7	0.94 (0.40-2.20)	7	1.02 (0.44-2.39)	
Anti-EA, titer‡								.13
< 640	525	64	Ref	45	Ref	42	Ref	
≥ 640	104	7	0.54 (0.24-1.22)	14	1.54 (0.80-2.96)	10	1.08 (0.52-2.24)	
EBNA-1/EBNA-2 ratio‡								.82
> 1.0	424	42	Ref	35	Ref	32	Ref	
≤ 1.0	205	29	1.52 (0.91-2.55)	24	1.29 (0.74-2.27)	20	1.21 (0.66-2.21)	

EBV indicates Epstein-Barr virus; NHL, non-Hodgkin lymphoma; DLBCL, diffuse large B-cell lymphoma; CLL/SLL, chronic lymphocytic leukemia/small lymphocytic lymphoma; FL, follicular lymphoma; EBNA, Epstein-Barr nuclear antigen; VCA, viral capsid antigen; EA, early antigen; RR, rate ratio; and CI, confidence interval.

*Unconditional logistic regression adjusting for the matching factors (sex/study, age, race, month and year of blood draw, fasting status).

†*P* for heterogeneity of effects.

‡Among EBV-seropositive subjects only.

titer was statistically significant ($P = .04$). There was no evidence of a linear trend in the associations between the other EBV antibodies and risk of CLL/SLL, DLBCL, or FL when evaluated on the base 2 logarithmic scale, and the corresponding tests for heterogeneity were nonsignificant (all *P* values for heterogeneity $> .20$; data not shown).

Discussion

In this prospective study of apparently immunocompetent men and women, we observed no associations between EBV serostatus; elevated antibody titers against EBNA-1, EBNA-2, VCA, or EA; or a low EBNA-1/EBNA-2 titer ratio and risk of NHL overall. These results are in contrast to several retrospective[15-17] and prospective[12-14] case-control studies that have reported positive associations between elevated EBV antibody titers and risk of NHL in nonimmunosuppressed populations. Specifically, Mueller et al found that NHL cases were more likely than controls to have elevated levels of IgG against VCA (RR, 2.5; 95% CI, 1.1-5.7) measured an average of 5 years before diagnosis.[12] These investigators also reported a nonsignificant lower risk of NHL associated with either high or low titers against the EBNA antigens.[12] Lehtinen et al[13] observed a nonsignificantly increased risk of NHL associated with elevated anti-EA IgG and anti–EBNA-1 IgG levels in a case-control study nested within a cohort of 39 000 Finnish adults; however, only 11 cases occurred during the 12 years of follow-up.[13] Rothman et al reported an elevated risk of NHL among persons who were seropositive for anti-EA IgG measured in a prospective analysis (OR, 2.6; 95% CI, 1.2-5.9).[14] Two retrospective case-control studies from Sweden also showed that NHL cases were more likely than controls to have elevated titers of IgG antibodies against EA and VCA, with RRs ranging from 1.6 to 2.4[15,17]; however, statistical significance was evident only for the effect of anti-EA titers above the median in the more recent of the 2 studies (OR, 2.4; 95% CI, 1.3-4.3).[17] Finally, a recent analysis in the EPILYMPH study found that "abnormal" EBV antibody reactivity profiles, as defined by immunoblot analysis, were associated with increased risk of all B-cell lymphomas (OR, 2.88; 95% CI, 2.16-3.85).[16]

In subgroup analyses, we observed a nonsignificant increased risk of NHL associated with elevated anti–EBNA-1 titer in women only. However, there was no difference by sex when considering the EBNA-1/EBNA-2 ratio, which may be a better indicator of an altered host response to EBV.[3] A positive association between elevated anti-VCA IgG and risk of NHL was suggested for men but not women. Interestingly, Mueller et al reported the opposite finding for anti-VCA IgG.[12] Although immune function is known to differ by sex,[30] we are not aware of any biologic reason why EBV antibody titers should be differentially related to lymphoma risk by sex. Moreover, there was limited evidence for statistical interaction; therefore, the sex-specific findings could be due to chance. Mueller et al also reported stronger associations between EBV antibody profiles and NHL risk with increasing age.[12] In the current study, we found no apparent differences by age group. In contrast to a prior report of differences in antibody titer associations by interval to diagnosis,[12] we found no conclusive evidence that a given EBV serologic pattern predicts longer- or shorter-term NHL risk.

Recent evidence suggests etiologic heterogeneity among NHL subtypes.[31] We were able to explore the association between pattern of EBV antibody response and risk of CLL/SLL, DLBCL, and FL. In these analyses, we observed suggestive evidence of an increased risk of CLL/SLL associated with elevated titers against EBNA-2 and VCA and an EBNA-1/EBNA-2 ratio ≤ 1.0. This EBV serology profile could reflect enhanced viral replication or relatively poor host control of infection.[3,8] By contrast, associations between EBV antibodies and risk of DLBCL or FL were largely null. We had no a priori hypotheses about which subtypes, if any, might be associated with a given EBV antibody pattern. Severe immune deficiency

Downloaded from http://ashpublications.org/blood/article-pdf/116/18/3547/1330722/zh804410003547.pdf by guest on 21 May 2024

appears to increase the risk of many types of NHL and particularly of NHL tumors in which latent EBV infection can be detected.[32] In contrast, recent studies of polymorphisms in genes involved in immune regulation (eg, tumor necrosis factor, interleukin-10) suggested that subclinical immune dysfunction may be more important in the development of DLBCL than CLL/SLL and FL.[31,33] Mueller et al reported no differences in effects of EBV serology for diffuse versus follicular histology.[12] Although based on small numbers, our suggestive findings of EBV antibody responses that are believed to be indicative of poorly controlled infection preceding the diagnosis of CLL/SLL are consistent with results from the recent case-control study by de Sanjosé et al[16] in which the strongest association with an abnormal immunoblot-defined pattern of EBV antibody reactivity was for CLL/SLL (OR, 2.96; 95% CI, 2.22-3.95). Compared with DLBCL and FL, which arise from germinal center or post–germinal center B cells, CLL/SLL is typically characterized by a more indolent disease process and is thought to arrive from naive or memory B cells.[34]

As discussed, the published literature on EBV antibodies and risk of NHL is somewhat inconsistent, with different antibody patterns reported to be associated with increased NHL risk by different studies. Possible reasons for discrepancies in results might include differences in laboratory assays, differences in study design (ie, retrospective vs prospective analyses), or limited statistical power due to relatively small study populations. Importantly, the associations may differ by histologic subtype of NHL.

If the observed associations between EBV serologic profile and NHL are indicative of a causal role for EBV in the pathogenesis of NHL, one proposed biologic mechanism for this effect would involve immune system dysregulation (eg, EBV-driven lymphoproliferation). As noted, severe immune deficiency is one of the strongest identified risk factors for NHL. It is plausible that a state of prolonged, subclinical immunosuppression or perturbations of immune function could also confer excess risk.[32] In support of this hypothesis, strong evidence of multiplicative interaction between serologic markers of EBV infection and elevated concentrations of organochlorines, which are known to be immunotoxic, has been reported in 2 studies.[14,15] EBV infection has also been causatively linked with risk of autoimmune diseases, such as systemic lupus erythematosus,[35] which itself carries a 5- to 8-fold increased risk of NHL.[36,37] Given these observations, it is possible that both autoimmune diseases and NHL could be uncommon consequences of EBV infection. However, whether EBV infection (with or without IM) induces an immunosuppressive state in some persons or whether EBV antibody pattern is a nonspecific marker of underlying immune dysfunction (eg, loss of immune control of latent infection) could not be evaluated in this study and remains an important question about lymphomagenesis. Lack of information about the timing of EBV infection or history of IM, which could help answer this question, is a potential limitation of this study. However, most people in the United States are infected with EBV before or during adolescence or young adulthood.[38] We found no clear differences in associations when stratifying by age or time to diagnosis.

A main strength of this study is its prospective nature. Because the development of NHL itself leads to altered immunity, the possibility that B-cell proliferation induces EBV reactivation, leading to altered antibody patterns, cannot be ruled out in retrospective studies. Only 3 prospective studies on the association between EBV serology and NHL in previously healthy persons have been published to date.[12-14] All 3 reported positive associations between elevated EBV antibodies and NHL, but the specific serologic patterns associated with risk differed among the studies. These investigations were also based on small numbers of cases, with 104 cases in the largest prospective study.[12] With >3 times that number of cases in the present analysis, we had a unique opportunity to evaluate possible differences in association of an EBV antibody pattern with NHL risk by major histologic subtype. Overall, we found no clear evidence that EBV serologic profile predicts risk of NHL in immunocompetent persons, with the possible exception of CLL/SLL. The overall lack of association, however, does not rule out a possible association of other NHL subtypes with EBV among susceptible subgroups. Evaluation of potential interactions between EBV infection and other environmental exposures, such as organochlorines, or polymorphisms in immune regulation genes, may help to clarify the association and to elucidate a biologic mechanism, if any.

Acknowledgments

We thank Annika Linde and staff in her laboratory at the Swedish Institute for Infectious Disease Control Department of Virology for performing the EBV antibody assays, Scott J. Rodig for his assistance with histologic subtyping, and Hans-Olov Adami for his scientific input and comments on an early draft of this manuscript.

This work was supported by the National Institutes of Health (CA098122, CA87969, CA34944, CA40360, HL26490, HL34595, and CA097193). K.A.B. was supported by the Training Program in Environmental Health Sciences (T32 ES007155) and by the Nutritional Epidemiology of Cancer Education and Career Development Program (R25 CA098566).

Authorship

Contribution: F.L. (principal investigator) designed the study and secured funding with S.M.Z. and J.C.A; K.A.B. performed statistical analyses with input from D.S., B.M.B., E.T.C., S.M.Z., and F.L.; J.C.A. determined histologic subtype; and K.A.B. wrote the first draft of the manuscript, which was critically revised and approved by all authors.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

Correspondence: Kimberly A. Bertrand, Department of Epidemiology, Harvard School of Public Health, 677 Huntington Ave, Boston, MA 02115; e-mail: kbertran@hsph.harvard.edu.

References

1. American Cancer Society. *Cancer Facts & Figures, 2009*. Atlanta, GA: American Cancer Society; 2009.
2. Kutok JL, Wang F. Spectrum of Epstein-Barr virus-associated diseases. *Annu Rev Pathol.* 2006; 1:375-404.
3. Henle W, Henle G, Andersson J, et al. Antibody responses to Epstein-Barr virus-determined nuclear antigen (EBNA)-1 and EBNA-2 in acute and chronic Epstein-Barr virus infection. *Proc Natl Acad Sci U S A.* 1987;84(2):570-574.
4. Young LS, Rickinson AB. Epstein-Barr virus: 40 years on. *Nat Rev Cancer.* 2004;4(10):757-768.
5. Babcock GJ, Decker LL, Volk M, Thorley-Lawson DA. EBV persistence in memory B cells in vivo. *Immunity.* 1998;9(3):395-404.
6. Cohen JI. Epstein-Barr virus infection. *N Engl J Med.* 2000;343(7):481-492.
7. Lennette ET, Rymo L, Yadav M, et al. Disease-related differences in antibody patterns against EBV-encoded nuclear antigens EBNA 1, EBNA 2

Downloaded from http://ashpublications.org/blood/article-pdf/116/18/3547/1330722/zh804410003547.pdf by guest on 21 May 2024

and EBNA 6. *Eur J Cancer.* 1993;29A(11):1584-1589.

8. Henle W, Henle G. Epstein-Barr virus-specific serology in immunologically compromised individuals. *Cancer Res.* 1981;41(11 Pt 1):4222-4225.
9. International Agency for Research on Cancer (IARC). *Epstein-Barr Virus and Kaposi's Sarcoma Herpesvirus/Human Herpesvirus 8.* Vol 70. Lyon, France: IARC; 1997.
10. Niedobitek G, Meru N, Delecluse HJ. Epstein-Barr virus infection and human malignancies. *Int J Exp Pathol.* 2001;82(3):149-170.
11. Mueller N. Overview of the epidemiology of malignancy in immune deficiency. *J Acquir Immune Defic Syndr.* 1999;21(suppl 1):S5-10.
12. Mueller N, Mohar A, Evans A, et al. Epstein-Barr virus antibody patterns preceding the diagnosis of non-Hodgkin's lymphoma. *Int J Cancer.* 1991; 49(3):387-393.
13. Lehtinen T, Lumio J, Dillner J, et al. Increased risk of malignant lymphoma indicated by elevated Epstein-Barr virus antibodies–a prospective study. *Cancer Causes Control.* 1993;4(3):187-193.
14. Rothman N, Cantor KP, Blair A, et al. A nested case-control study of non-Hodgkin lymphoma and serum organochlorine residues. *Lancet.* 1997;350(9073):240-244.
15. Hardell E, Eriksson M, Lindstrom G, et al. Case-control study on concentrations of organohalogen compounds and titers of antibodies to Epstein-Barr virus antigens in the etiology of non-Hodgkin lymphoma. *Leuk Lymphoma.* 2001;42(4):619-629.
16. de Sanjose S, Bosch R, Schouten T, et al. Epstein-Barr virus infection and risk of lymphoma: immunoblot analysis of antibody responses against EBV-related proteins in a large series of lymphoma subjects and matched controls. *Int J Cancer.* 2007;121(8):1806-1812.
17. Hardell K, Carlberg M, Hardell L, et al. Concentrations of organohalogen compounds and titres of antibodies to Epstein-Barr virus antigens and the risk for non-Hodgkin lymphoma. *Oncol Rep.* 2009;21(6):1567-1576.
18. Swerdlow SH, Campo E, Harris NL, et al. eds. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues.* Lyon, France: International Agency for Research on Cancer (IARC) Press; 2008.
19. Steering Committee of the Physicians' Health Study Research Group. Final report on the aspirin component of the ongoing Physicians' Health Study. *N Engl J Med.* 1989;321(3):129-135.
20. Ma J, Li H, Giovannucci E, et al. Prediagnostic body-mass index, plasma C-peptide concentration, and prostate cancer-specific mortality in men with prostate cancer: a long-term survival analysis. *Lancet Oncol.* 2008;9(11):1039-1047.
21. Rich-Edwards JW, Corsano KA, Stampfer MJ. Test of the National Death Index and Equifax Nationwide Death Search. *Am J Epidemiol.* 1994; 140:1016-1019.
22. Linde A, Andersson J, Lundgren G, Wahren B. Subclass reactivity to Epstein-Barr virus capsid antigen in primary and reactivated EBV infections. *J Med Virol.* 1987;21(2):109-121.
23. Linde A, Kallin B, Dillner J, et al. Evaluation of enzyme-linked immunosorbent assays with two synthetic peptides of Epstein-Barr virus for diagnosis of infectious mononucleosis. *J Infect Dis.* 1990;161(5):903-909.
24. Evans AS, Mueller N. Epstein-Barr virus and malignant lymphomas. In: Evans A, Kaslow R, eds. *Viral Infections of Humans: Epidemiology and Control.* New York, NY: Plenum Press; 1997:895-933.
25. Durrleman S, Simon R. Flexible regression models with cubic splines. *Stat Med.* 1989;8(5):551-561.
26. Larsson SC, Wolk A. Obesity and risk of non-Hodgkin's lymphoma: a meta-analysis. *Int J Cancer.* 2007;121(7):1564-1570.
27. Morton LM, Hartge P, Holford TR, et al. Cigarette smoking and risk of non-Hodgkin lymphoma: a pooled analysis from the International Lymphoma Epidemiology Consortium (InterLymph). *Cancer Epidemiol Biomarkers Prev.* 2005;14(4):925-933.
28. Zhang S, Hunter DJ, Rosner BA, et al. Dietary fat and protein in relation to risk of non-Hodgkin's lymphoma among women. *J Natl Cancer Inst.* 1999;91(20):1751-1758.
29. Bertrand KA, Spiegelman D, Aster JC, et al. Plasma organochlorine levels and risk of non-Hodgkin lymphoma in a cohort of men. *Epidemiology.* 2010;21(2):172-180.
30. Fish EN. The X-files in immunity: sex-based differences predispose immune responses. *Nat Rev Immunol.* 2008;8(9):737-744.
31. Morton LM, Wang SS, Cozen W, et al. Etiologic heterogeneity among non-Hodgkin lymphoma subtypes. *Blood.* 2008;112(13):5150-5160.
32. Grulich AE, Vajdic CM, Cozen W. Altered immunity as a risk factor for non-Hodgkin lymphoma. *Cancer Epidemiol Biomarkers Prev.* 2007;16(3): 405-408.
33. Rothman N, Skibola CF, Wang SS, et al. Genetic variation in TNF and IL10 and risk of non-Hodgkin lymphoma: a report from the InterLymph Consortium. *Lancet Oncol.* 2006;7(1):27-38.
34. Jaffe ES, Harris NL, Stein H, Vardiman JW, eds. *WHO Classification of Tumours: Pathology and Genetics of Tumours of Haematopoietic and Lymphoid Tissues.* Lyon, France: International Agency for Research on Cancer (IARC) Press; 2001.
35. James JA, Neas BR, Moser KL, et al. Systemic lupus erythematosus in adults is associated with previous Epstein-Barr virus exposure. *Arthritis Rheum.* 2001;44(5):1122-1126.
36. Mellemkjaer L, Andersen V, Linet MS, Gridley G, Hoover R, Olsen JH. Non-Hodgkin's lymphoma and other cancers among a cohort of patients with systemic lupus erythematosus. *Arthritis Rheum.* 1997;40(4):761-768.
37. Vineis P, Crosignani P, Sacerdote C, et al. Haematopoietic cancer and medical history: a multicentre case control study. *J Epidemiol Community Health.* 2000;54(6):431-436.
38. Hsu JL, Glaser SL. Epstein-Barr virus-associated malignancies: epidemiologic patterns and etiologic implications. *Crit Rev Oncol Hematol.* 2000; 34(1):27-53.

Downloaded from http://ashpublications.org/blood/article-pdf/116/18/3547/1330722/zh804410003547.pdf by guest on 21 May 2024