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Association of Epstein-Barr Virus With Systemic Lupus Erythematosus

Effect Modification by Race, Age, and Cytotoxic T Lymphocyte–Associated Antigen 4 Genotype

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Objective. **Epstein-Barr virus (EBV) is hypothesized to play a role in the development of systemic lupus erythematosus (SLE). Cytotoxic T lymphocyte–associated antigen 4 (CTLA-4) is important in regulating T cell–mediated immunity, encompassing the first line of response to viral infections, and genetic variation in CTLA-4 has been associated with SLE. This study examined the seroprevalence of EBV in a population-based study of SLE patients from the southeastern United States, and potential interactions with CTLA-4 polymorphisms were assessed.**

Methods. **Cases comprised 230 subjects recently diagnosed as having SLE (144 African American and 86 white) from university and community-based clinics, and controls comprised 276 age-, sex-, and state-matched subjects (72 African American and 204 white) recruited from driver's license registries. Antibodies to EBV capsid antigen were determined by enzyme-linked immunosorbent assay, with results expressed as positive or negative using the international standardized ratio (ISR) (a ratio of the sample absorbance to a known standard). CTLA-4 genotypes were identified by polymerase chain reaction–based methods.**

Results. **In African Americans, EBV-IgA seroprevalence was strongly associated with SLE (odds ratio [OR] 5.6, 95% confidence interval [95% CI] 3.0–10.6). In whites, the modest association of SLE with EBV-IgA (OR 1.6) was modified by age, in that the strongest association was observed in those older than age 50 years (OR 4.1, 95% CI 1.6–10.4). The seroprevalence of EBV-IgM and that of EBV-IgG were not associated with SLE. Higher EBV-IgG absorbance ratios were observed in SLE patients, with a significant dose response across units of the ISR in African Americans ($P < 0.0001$). Allelic variation in the CTLA-4 gene promoter (−1661A/G) significantly modified the association between SLE and EBV-IgA ($P = 0.03$), with a stronger association among those with the −1661AA genotype.**

Conclusion. **These findings suggest that repeated or reactivated EBV infection, which results in increased EBV-IgA seroprevalence and higher IgG antibody titers, may be associated with SLE, and that the CTLA-4 genotype influences immune responsiveness to EBV in SLE patients. The observed patterns of effect modification by race, age, and CTLA-4 genotype should be examined in other studies and may help frame new hypotheses regarding the role of EBV in SLE etiology.**

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Epstein-Barr virus (EBV) is a herpes virus that is present in the majority of adults (>90%). The virus initially infects epithelial and B cells, which is followed by life-long latent infection of B cells with occasional reactivation and productive cycles of viral replication (1). EBV has long been hypothesized to play a role in the etiology of systemic lupus erythematosus (SLE) (2). This hypothesis is based, in part, on the idea that loss of EBV latency in SLE patients may be related to chronic immune stimulation, as well as the observation that EBV may promote antibodies that crossreact with self antigens. Two recent studies have described an association between having a history of EBV infection and having SLE, reflected by the presence of EBV-IgG antibodies to viral capsid antigen (VCA), both in pediatric and adolescent patients and in adult patients from multiplex SLE families (3,4). Given the ubiquitous nature of EBV as an exposure risk in the general population, unraveling the role of EBV in SLE may require understanding the role of other factors, such as genetic polymorphisms, that may be related to immune responsiveness to EBV.

Maintaining EBV infection in a latent state primarily depends on T cell–mediated immunity (5). Cytotoxic T lymphocyte–associated antigen 4 (CTLA-4), which is expressed on activated T cells, plays a role in regulating the T cell response and is involved in immune tolerance mechanisms (6). Polymorphisms have been identified in the promoter region of the human CTLA-4 gene (−1722T/C, −1661A/G, −318C/T) and exon 1 (+49G/A), some of which are related to differences in CTLA-4 expression or have been associated with susceptibility to autoimmune diseases (7,8), including SLE (9). Given the role of CTLA-4 in regulating the immune response, we hypothesized that the CTLA-4 genotype might modify the association between the EBV-specific immune response and SLE.

Genetic variation in cytokine genes has also been associated both with the presence of EBV antibodies (10–12) and with SLE (13,14). We previously reported associations between SLE and allelic variation in interleukin-1 (IL-1) (IL-1α −889C/T, IL-1β −511C/T) and tumor necrosis factor α (TNFα) (−308A/G) in a population-based study of SLE in the southeastern United States (15,16). Thus, we also hypothesized that these loci might represent additional steps along the pathway that influences immune responsiveness to EBV in SLE.

In the present study, we determined the prevalence of antibodies to EBV VCA in a sample of recently diagnosed SLE patients and population-based controls. Specifically, we examined the presence of IgG antibodies to EBV VCA, which can persist for decades and reflect past exposure to EBV. We also determined the prevalence of EBV-IgA antibodies, which are thought to indicate reactivation or reinfection with EBV, especially in the presence of elevated IgG antibody titers (17). The examination of EBV-IgA seroprevalence in addition to IgG antibody levels allowed the investigation of the variation in immune response to EBV among the general SLE patient population, overcoming, in part, the limitation of high overall EBV-IgG seroprevalence. We also examined the prevalence of antibodies to other herpes viruses, herpes simplex virus 1 (HSV-1) and cytomegalovirus (CMV), as a comparison, considering the possibility that SLE patients could have nonspecific dysregulation of immune control of other latent viral infections in addition to EBV. Finally, we examined whether patient characteristics, such as race and age, time since diagnosis, or genetic variation in CTLA-4, IL-1, and TNFα could modify the association between EBV antibodies and SLE.

PATIENTS AND METHODS

Study sample. Subjects were from the Carolina Lupus Study, a population-based case–control study of SLE based in 60 contiguous counties in North Carolina and South Carolina. Cases were subjects recently diagnosed (January 1995 to July 1999) as having SLE, who were identified through 4 university-based and 30 community-based practices. A patient was considered eligible for the study if he or she met the American College of Rheumatology diagnostic criteria for SLE (18), was older than age 18 years, and spoke English. The final sample consisted of 265 cases, which reflected a final enrollment and participation of 93% of the referred patients. Of the eligible 285 patient referrals, 6 refused screening and 14 declined to participate. About half of the enrolled patients were from community-based practices. Patients were enrolled in the study within a median of 13 months after diagnosis. Ninety percent of the patients were women and 60% were African American.

Controls were subjects randomly selected from the states' driver's license agencies. These subjects were frequency-matched to the patients on age (by 5-year age groups), sex, and state. Contact rates and overall response rates were lower for controls. The final sample comprised 355 control subjects (75% of the screened and eligible individuals). Of 1,873 potential controls originally selected from driver's license registries, 911 were ineligible due to invalid address or telephone number, death, or having moved out of the study area. Of the remaining 962 potential controls who were contacted for telephone screening, 163 (17%) refused screening, 195 (20%) were screened but ineligible, 120 (12%) were eligible but declined to participate, and 129 (13%) were eligible but enrollment was deferred because sample size within their strata had been reached. Thirty percent of enrolled control subjects were African American, which is similar to the racial distribution of the study area based on census estimates.

Matching by race can improve the efficiency (in terms of cost-effectiveness) of a study but is not necessary to produce unbiased estimates of effects (19).

The present study of EBV seroprevalence was limited to African American and white subjects. Blood specimens were available from 92% of patients and 82% of controls, resulting in a final sample size of 230 cases (144 African American and 86 white) and 276 controls (72 African American and 204 white). Clinical features of the patients (up to 6 months after diagnosis) were abstracted from medical records, as previously described (20).

Viral antibody determinations. History of infection was assessed for 3 common latent herpes viruses: EBV, HSV-1, and CMV. Serum antibodies (IgG, IgM, and IgA) to these viruses were measured by enzyme-linked immunosorbent assay (ELISA) (Hope Laboratories, Belmont, CA). Optical density values were converted into standardized units (the international standardized ratio [ISR]; a ratio of the absorbencies to a known calibration standard), as recommended by the manufacturer. Samples were then assigned as antibody positive (ISR $\geq$1), negative (ISR $\leq$0.90), or equivocal (ISR 0.91–0.99; defined as samples that were tested twice, with neither reading yielding positive or negative findings). Positive and negative controls were included on each plate in addition to the calibration standards. For comparisons of EBV-IgG antibody concentrations, absorbance ratios were considered on a continuous scale (e.g., in controls, median ISR 1.88, mean 2.15, SD 1.01; in cases, median ISR 2.92, mean 2.88, SD 1.16). In addition, we used a 4-level ordinal variable to compare cases and controls for IgG concentrations, based on the ISR distribution among controls using race-specific cutoff points, or quartiles (Q1–Q4): African Americans, ISR Q1 0.31–1.54, Q2 1.55–1.99, Q3 2.0–2.85, Q4 $\geq$2.86; whites, ISR Q1 0.04–1.40, Q2 1.41–1.85, Q3 1.86–2.61, Q4 $\geq$2.62. The absorbency of the specimens was analyzed without knowledge of case status, and there was no significant difference in the distribution of cases and controls by batch.

Genotyping. CTLA-4 genotypes were assessed by polymerase chain reaction–restriction fragment-length polymorphism (PCR-RFLP) analysis at 4 loci (-1722, -1661, -319, and $+49$), as previously described (9,21–23). Genotypes were in Hardy-Weinberg equilibrium. Significant racial differences in genotype frequency were seen between African American and white controls at -1722 and between African American and white cases at -1661 and -318 (24). Polymorphisms in IL-1 (IL-1α -889, IL-1β -511) and TNF (TNFα -308) were also assessed by PCR-RFLP analysis, as previously described (15,16). Genotype frequencies were in Hardy-Weinberg equilibrium, and were similar between African American and white controls, except for a significant difference at IL-1β -511 (15).

Statistical analysis. All analyses were conducted using SAS software, version 8.2 (Cary, NC). The frequency of viral antibodies in cases and controls was evaluated in parallel analyses for African Americans and whites. Racial differences in antibody frequency were assessed separately for cases and controls. The associations between SLE and the presence of EBV, HSV-1, and CMV antibodies were evaluated in race-stratified analyses as follows. Logistic regression models were constructed with the inclusion of each viral antibody measure separately (e.g., EBV-IgG, -IgM, and -IgA). Multivariate models were also constructed, and these included variables for the ELISA plate (7 batches), season, matching factors (age, sex, and state), and education (4 levels). Confounding was evaluated by a change in the beta coefficient for the main effect when a potential confounder was added to the model, with a difference of greater than or equal to 10% considered to be significant. None of the factors evaluated were found to confound the EBV–SLE associations, and thus were not included in the final analyses. Models were also run to assess for potential confounding between EBV-IgG and -IgA antibodies or CMV- and EBV-IgA antibodies.

Effect modification of the seroprevalence data by race was evaluated using interaction terms for each of the antibodies with race (African American and white). Statistical significance of the interactions was assessed by comparing the difference in the -2 log likelihood values for models with and those without the interaction terms; interactions were considered suggestive if P values were less than 0.20, and significant if P values were less than 0.10. To evaluate potential effect modification, models of the EBV–SLE association were also evaluated in analyses stratified by age as well as CTLA-4 and cytokine genotypes. The apparent effect modification of the SLE–EBV-IgA association by age, seen in stratified analyses, was also assessed using age as a continuous variable in the interaction term (age in years$*$IgA). Interaction terms were also constructed for race$*$IgA and CTLA-4 genotype$*$IgA. A complete model was constructed to evaluate the interaction of the CTLA-4 -1661 polymorphism and EBV-IgA antibodies with respect to SLE, while taking into account previously described interactions of the CTLA-4 -1661 polymorphism with age and race (24). The best-fit model after both forward and backward elimination included the following variables: age, sex, state, race, EBV-IgA, race$*$EBV-IgA, older age$*$EBV-IgA, CTLA-4 -1661AA, CTLA-4 $-1661*$race, CTLA-4 $-1661*$EBV-IgA (difference in the -2 log likelihood for models with and those without CTLA-4$*$IgA; P for interaction = 0.0309).

To evaluate whether other aspects of having SLE were associated with EBV-IgA antibodies, we examined patterns of seroprevalence with respect to time since diagnosis and proteinuria. Among the African American and white patients, 89 (61%) and 61 (71%), respectively, had blood specimens obtained within 1 year of diagnosis. Of the African American patients, 43 were diagnosed as having proteinuria based on a review of the medical records. The numbers of whites with proteinuria (n = 11) were too small to assess, and therefore these analyses were performed in African Americans only.

RESULTS

EBV–SLE association. Table 1 lists the prevalence of IgG, IgM, and IgA antibodies to EBV, HSV-1, and CMV with respect to the presence or absence of SLE in African Americans and whites. African Americans had a significantly higher prevalence of EBV-IgG VCA antibodies compared with whites, both among the SLE cases and among the controls. Moreover, African

Table 1. Distribution of EBV, CMV, and HSV-1 antibodies in patients with systemic lupus erythematosus (SLE) and controls, and differences by race in viral seroprevalence and association with SLE*

	Seroprevalence to latent viruses						Difference in seroprevalence by race in cases/controls, P†	Effect modification by race, P‡
	African American			White				
	Cases (n = 144), no. (%)	Controls (n = 72), no. (%)	OR (95% CI)	Cases (n = 86), no. (%)	Controls (n = 204), no. (%)	OR (95% CI)		
EBV								
IgG								
Pos.	142 (99)	71 (99)	1.0 (0.09–11.2)	78 (91)	185 (91)	1.0 (0.42–2.4)	0.007/0.03	0.99
Neg.	2 (1)	1 (1)		8 (9)	19 (9)			
Equivocal	0	0		0	0			
IgM								
Pos.	2 (1)	0	NC	1 (1)	1 (1)	NC	1.0/0.55	NC
Neg.	142 (99)	72 (100)		85 (99)	203 (99)			
Equivocal	0	0		0	0			
IgA								
Pos.	95 (66)	18 (25)	5.6 (3.0–10.6)§	37 (43)	66 (32)	1.6 (0.94–2.7)	<0.001/0.28	0.0027
Neg.	49 (34)	52 (72)		48 (56)	136 (66)			
Equivocal	0	2 (3)		1 (1)	2 (1)			
HSV-1								
IgG								
Pos.	127 (88)	61 (82)	1.4 (0.59–3.1)	60 (70)	138 (68)	1.1 (0.62–1.9)	<0.001/0.014	0.65
Neg.	17 (12)	11 (15)		26 (30)	64 (31)			
Equivocal	0	0		0	2 (1)			
IgM								
Pos.	12 (8)	4 (6)	1.6 (0.48–5.0)	8 (9)	16 (8)	1.2 (0.51–3.0)	0.80/0.71	0.75
Neg.	130 (90)	68 (94)		76 (88)	187 (92)			
Equivocal	2 (1)	0		2 (2)	1 (0)			
IgA								
Pos.	130 (90)	62 (86)	1.5 (0.63–2.6)	65 (76)	149 (73)	1.1 (0.64–2.0)	0.004/0.025	0.61
Neg.	14 (10)	10 (14)		21 (24)	55 (27)			
Equivocal	0	0		0	0			
CMV								
IgG								
Pos.	116 (81)	57 (79)	1.1 (0.54–2.2)	47 (55)	116 (57)	0.91 (0.55–1.5)	<0.001/0.014	0.69
Neg.	28 (19)	15 (21)		39 (45)	88 (43)			
Equivocal	0	0		0	0			
IgM								
Pos.	16 (11)	6 (8)	1.4 (0.51–3.7)	8 (9)	10 (5)	2.0 (0.76–5.2)	0.80/0.71	0.60
Neg.	127 (88)	66 (92)		78 (91)	194 (95)			
Equivocal	1 (1)	0		0	0			
IgA								
Pos.	126 (87)	52 (72)	2.6 (1.2–5.3)¶	51 (59)	116 (57)	1.2 (0.68–1.9)	0.004/0.025	0.034
Neg.	18 (13)	19 (26)		33 (38)	87 (42)			
Equivocal	0	1 (1)		2 (2)	1 (0)			

* Values are the odds ratio (OR) and 95% confidence interval (95% CI) comparing subjects who were seropositive (Pos.) for antibodies with seronegative (Neg.) subjects, and excluding equivocal samples. EBV = Epstein-Barr virus; CMV = cytomegalovirus; HSV-1 = herpes simplex virus 1; NC = not calculated due to small numbers of seropositive individuals in these subgroups. Models were not confounded by matching factors, smoking, or education; thus, models shown are unadjusted.

† Difference between positive and negative, not including equivocal ratings. Equivocal = tested twice with neither positive nor negative finding.

‡ Test for interaction between race and seroprevalence, with P value shown for difference in the −2 log likelihoods. Interaction term not tested for EBV- IgM due to small numbers of seropositive individuals in this group.

§ $P < 0.0001$, comparing EBV-IgA positive with EBV-IgA negative.

¶ $P = 0.01$, comparing CMV-IgA positive with CMV-IgA negative.

Table 2. Distribution of the EBV-IgG absorbance ratio in patients with systemic lupus erythematosus and controls*

	African American			White		
Quartile	Cases (n = 144), no. (%)	Controls (n = 72), no. (%)	OR (95% CI)†	Cases (n = 86), no. (%)	Controls (n = 204), no. (%)	OR (95% CI)†
Q1 (lowest)	7 (5)	17 (24)	Referent	11 (13)	50 (25)	Referent
Q2	16 (11)	18 (25)	1.8 (0.57–5.9)	20 (23)	50 (25)	1.7 (0.75–4.0)
Q3	32 (22)	19 (25)	3.8 (1.3–11.5)	23 (27)	52 (25)	1.8 (0.79–4.1)
Q4 (highest)	89 (62)	18 (25)	8.9 (3.0–26.3)‡	32 (37)	52 (25)	2.6 (1.1–5.8)‡

* EBV-IgG absorbance ratios were considered on a continuous scale (e.g., in controls, the median international standardized ratio [ISR] was 1.88 with a mean of 2.15 and SD of 1.01; in cases, the median ISR was 2.92, with a mean of 2.88 and SD of 1.16), and were also considered using a 4-level ordinal variable based on the ISR distribution in controls and using race-specific cutoff points (African Americans ISR Q1 0.31–1.54, Q2 1.55–1.99, Q3 2.0–2.85, Q4 ≥2.86; whites ISR Q1 0.04–1.40, Q2 1.41–1.85, Q3 1.86–2.61, Q4 ≥2.62). See Table 1 for other definitions.
† The OR and 95% CI was adjusted for EBV-IgA seroprevalence.
‡ To examine dose response, the OR was generated for a continuous variable (ISR ratio). For African Americans, this OR was 2.0 ($P < 0.0001$) and for whites, this OR was 1.2 ($P = 0.12$), per unit increase in ISR.

Americans had a higher prevalence of EBV-IgA antibodies among the SLE cases. SLE was not associated with an overall history of EBV infection, as indicated by the presence of EBV-IgG VCA antibodies, nor was it associated with recent EBV infection, as represented by the presence of EBV-IgM antibodies. However, SLE and the seroprevalence of EBV-IgA antibodies were strongly associated in African Americans (odds ratio [OR] 5.6) and modestly associated in whites (OR 1.6). This difference reflected a significant interaction by race on the association between SLE and EBV-IgA. The presence of EBV-IgA antibodies was common in those with IgG antibodies (52% of African Americans and 35% of whites with EBV-IgG antibodies also had IgA antibodies). This did not confound the observed associations between EBV-IgA and SLE, however, since there was no independent association of overall seroprevalence of EBV-IgG with SLE.

The patterns of racial differences in HSV-1 and CMV seroprevalence were similar to those seen for EBV, with a higher IgG and IgA seroprevalence in African Americans (Table 1). Neither HSV-1 antibodies (IgG, IgM, or IgA) nor CMV-IgG and -IgM antibodies were associated with SLE. In African Americans, but not whites, CMV-IgA seroprevalence was modestly associated with SLE (OR 2.6). EBV-IgG and -IgA antibodies were associated with both IgG and IgA antibodies for CMV and HSV-1 among the SLE cases (results not shown). However, the association between SLE and EBV-IgA was not confounded by the presence of CMV-IgA antibodies (CMV-IgA adjusted OR for the SLE–EBV-IgA association 5.3, 95% CI 2.8–10.1 in African Americans, and adjusted OR 1.6, 95% CI 0.98–2.9 in whites). Likewise, in African Americans, the association of SLE with CMV-IgA was scarcely changed after adjusting for EBV-IgA seroprevalence (OR 2.4, 95% CI 1.1–5.2).

Table 2 shows the association of SLE with EBV-IgG absorbance ratios (expressed as ISRs) across quartiles, adjusted for EBV-IgA seroprevalence. EBV-IgG ISRs were higher in SLE patients: cases were more likely to be in the highest quartile of EBV-IgG ISR than were controls (OR 8.9 for African Americans, and OR 2.6 for whites). African American cases in the highest quartile of EBV-IgG antibodies were more likely to be EBV-IgA antibody positive than were African American controls (71% versus 33%; $P = 0.0025$). In whites, a similar, although less pronounced, increase in EBV-IgA seroprevalence was seen among white cases in the highest IgG quartile compared with white controls (51% versus 24%; $P = 0.15$). The associations of EBV-IgG with SLE in the highest quartile were independent of the association of SLE with EBV-IgA antibodies in whites, but the unadjusted ORs for EBV-IgG in African Americans were somewhat higher than the IgA-adjusted values (results not shown) (unadjusted OR 12.0, 4.1, and 2.2 for African Americans in ISR quartiles 4, 3, and 2, respectively, compared with the lowest quartile).

To examine the possibility of a dose response, ORs were also generated for the continuous EBV-IgG ISR values. For African Americans, there was a significant dose response across units of the ISR (OR 2.0, $P < 0.0001$) that was less apparent in whites (OR 1.2, $P = 0.12$).

Interaction of EBV-IgA seroprevalence with race and age. The association of EBV seroprevalence with SLE appeared to be modified by age (Table 3). There was an inverse association between the presence of

Table 3. Prevalence and association between systemic lupus erythematosus (SLE) and EBV-IgG and -IgA antibodies, stratified by age*

	African American			White		
	Cases (n = 144)	Controls (n = 72)	OR (95% CI)†	Cases (n = 86)	Controls (n = 204)	OR (95% CI)†
EBV-IgG‡						
Age <35 years			NC			0.3 (0.1–1.0)
No.	74	34		32	79	
% seropositive	99	100		78	92	
Age ≥35 years			1.9 (0.1–30.7)			6.2 (0.8–48.2)
No.	70	38		54	125	
% seropositive	99	97		98	90	
EBV-IgA§						
Age <35 years			4.5 (1.8–11.2)			1.0 (0.2–4.6)
No.	74	34		32	79	
% seropositive	58	24		26	26	
Age 35–50 years			5.7 (1.9–16.7)			0.9 (0.3–2.2)
No.	45	26		24	64	
% seropositive	69	27		38	41	
Age >50 years			14.0 (2.5–76.9)			4.1 (1.6–10.4)
No.	25	12		30	61	
% seropositive	84	25		67	32	

* Except where indicated otherwise, numbers and percentages represent sample size and seroprevalence, respectively, in each age subgroup. The 2 higher age categories were combined for IgG because there were too few individuals over age 50 years who were negative for EBV-IgG antibodies to analyze separately. See Table 1 for other definitions.

† ORs and 95% CI are not adjusted for covariates. The OR was not calculated (NC) for EBV-IgG in young African Americans due to small numbers of seronegative individuals in this subgroup. The referent is seronegative individuals.

‡ Significant inverse association for IgG antibodies in those under age 35 years in combined analysis (African Americans and whites), controlling for race, matching factors, and education (OR 0.25, 95% CI 0.08–0.83, $P = 0.024$). Significant interaction for IgG status and age (as a continuous variable) with respect to SLE in whites ($P = 0.043$), but not African Americans ($P = 0.70$).

§ Significant interaction for IgA status and age (as a continuous variable) with respect to SLE in whites ($P = 0.009$), but not African Americans ($P = 0.63$).

EBV-IgG antibodies and SLE in both African Americans and whites under age 35 years (combined analysis controlling for race, OR 0.25, 95% CI 0.08–0.83), and there was a trend toward a positive association among older whites (OR 6.2). The modest association of SLE with EBV-IgA in whites (OR 1.6) was considerably stronger for those older than age 50 years (OR 4.1). This represented a significant interaction ($P = 0.009$) for EBV-IgA and age, modeled as a continuous variable in whites. A stronger association with EBV-IgA antibodies (OR 14.0) was also seen in African Americans older than age 50 years compared with those under age 35 years (OR 4.5), although this interaction was not significant.

We also examined EBV seroprevalence with respect to time since diagnosis, to evaluate whether other aspects of having SLE were associated with EBV-IgA antibodies (results not shown). In African American patients, the prevalence of EBV-IgA antibodies decreased somewhat with time since diagnosis (70% with EBV-IgA seropositivity among those sampled within 1 year of diagnosis versus 56% among those sampled at 3–5 years postdiagnosis), but the difference was not significant. In white patients, there was little evidence of a difference in EBV-IgA seroprevalence over time since diagnosis.

We also assessed whether there were differences in the SLE–EBV-IgA association for patients with and those without proteinuria, a possible indicator of medication use and severity. African American patients with proteinuria were less likely to be EBV-IgA seropositive (53% versus 71% of African American patients without proteinuria), with an inverse association between EBV-IgA seroprevalence and proteinuria compared with African American controls (OR 0.5, $P = 0.03$). Too few whites had renal involvement to perform the analysis in this subgroup.

Interaction of EBV-IgA seroprevalence and CTLA-4 genotype. Table 4 shows the association between SLE and EBV-IgA stratified by CTLA-4, IL-1,

Table 4. Association of EBV-IgA seroprevalence with systemic lupus erythematosus, stratified by CTLA-4 and cytokine genotypes*

Locus	African American			White		
	Cases (n = 144)	Controls (n = 72)	OR (95% CI)	Cases (n = 86)	Controls (n = 204)	OR (95% CI)
CTLA-4 −1661†						
AA			8.4 (3.6–10.5)			2.0 (1.1–3.7)
No.	78	45		62	127	
% seropositive	73	24		48	32	
AG + GG			3.3 (1.2–9.0)			0.8 (0.3–2.2)
No.	66	24		22	73	
% seropositive	57	29		27	33	
IL-1α −889						
CC			4.5 (1.4–20.0)			1.2 (0.6–2.6)
No.	62	17		43	68	
% seropositive	71	35		44	39	
CT + TT			5.6 (2.6–12.3)			1.9 (0.9–3.8)
No.	82	53		42	132	
% seropositive	62	22		43	29	
IL-1β −511						
CC			5.2 (1.3–20.5)			1.3 (0.6–2.9)
No.	23	20		41	88	
% seropositive	57	20		42	35	
CT + TT			5.4 (2.6–11.2)			1.9 (0.9–3.9)
No.	121	50		44	112	
% seropositive	68	28		45	31	
TNFα −308						
AA			5.4 (2.6–11.2)			1.7 (0.9–3.5)
No.	108	50		41	138	
% seropositive	68	28		46	33	
AG + GG			6.3 (1.7–22.7)			1.6 (0.7–3.6)
No.	36	20		44	63	
% seropositive	61	20		41	30	

* Except where indicated otherwise, numbers and percentages represent sample size and seroprevalence of EBV-IgA antibodies, respectively. Genotypes not available were as follows: for cytotoxic T lymphocyte–associated antigen 4 (CTLA-4) (2 African American controls, 2 white cases, 4 white controls), for interleukin-1α (IL-1α) −889 (2 African American controls, 1 white case, 4 white controls), for IL-1β −511 (2 African American controls, 2 white cases, 4 white controls), and for tumor necrosis factor α (TNFα) −308 (2 African American controls, 1 white case, 3 white controls). See Table 1 for other definitions.
† P for interaction = 0.03, in combined analysis of African Americans and whites.

and TNFα genotypes. EBV-IgA seroprevalence was higher in both African American and white SLE patients with the CTLA-4 −1661AA genotype compared with those who carried the G allele (73% versus 57% in African Americans and 48% versus 27% in whites), but there was no difference among controls. This resulted in stronger associations of EBV-IgA with SLE among those with the CTLA-4 −1661AA genotype (OR 8.4 in African Americans and OR 2.0 in whites) compared with carriers of the G allele (OR 3.3 in African Americans and OR 0.8 in whites).

A combined analysis was designed to test for the interaction of the −1661 polymorphism and EBV-IgA antibodies with respect to SLE, taking into account interactions between EBV-IgA, race, and age and the previously described interactions between CTLA-4 −1661 genotype, race, and age with respect to SLE (24). The best-fit model continued to show a significant interaction between CTLA-4 genotype and EBV-IgA with respect to SLE (interaction $P = 0.0309$) even after accounting for these other interactions. There were some differences in the association of EBV-IgA and SLE in analyses stratified by the other CTLA-4 loci (interactions not significant) (results not shown), but there was no evidence of effect modification by cytokine genotypes. Subsequent analyses revealed no significant differences in the associations of SLE with CMV-IgA or HSV-1–IgA by CTLA-4 genotype (results not shown).

DISCUSSION

In this population-based study, we observed a strong association between SLE and the presence of

EBV-IgA antibodies to VCA in African Americans and older whites. Although we did not observe an association between SLE and ever having a history of EBV infection, as reflected by overall IgG seroprevalence, SLE patients were more likely to have higher EBV-IgG titers, as reflected by the ISR values, in comparison with controls. The presence of EBV-IgG antibodies to VCA, generated during EBV replication, can persist for decades and thus can reflect any past exposure to EBV. In contrast, reactivation or reinfection with EBV is thought to cause increased production of EBV-IgA antibodies, especially in the presence of elevated EBV-IgG antibodies (17). Most patients with serologic evidence of EBV reactivation, however, do not express detectable levels of IgM capsid antibodies (25). Therefore, although we did not observe an association of markers of past (IgG) or recent (IgM) EBV infection with SLE, the findings of an association with IgA and high IgG titers suggest that reactivated or repeated infection with EBV may be associated with the development of SLE.

We did not observe a generalized pattern of association between SLE and antibodies to other latent viral infections, except for CMV-IgA, which was also associated with SLE among African Americans. The lack of a similar HSV-1–IgA association with SLE suggests that these results do not reflect a generalized phenomenon of B cell hyperactivity. Different factors may contribute to loss of latency for CMV and HSV-1 compared with EBV (26), since the viruses infect different cell types and have different patterns of replication. In case reports (27,28), CMV infection has been associated with the development of SLE. In the present study, CMV-IgM antibodies were slightly more common among SLE patients than among controls, although this trend was not significant in the combined analysis.

Two previous studies have demonstrated an association between the overall prevalence of IgG antibodies to EBV VCA and SLE (3,4). One factor that could account for the lack of replication of these findings in the present study is the difference in the patient populations. The patient samples in the studies that reported an EBV-IgG–SLE association included childhood (3) and familial SLE (4), which are 2 groups of subjects likely to have higher underlying susceptibility to SLE. These groups may provide a more sensitive indicator of the association of EBV infection with SLE if, for example, some of the putative susceptibility genes conferred increased risk of SLE through their influence on EBV immune responsiveness. In the present study, cases represented the general SLE patient population in the study area, including 50% from community-based practices, a wide age range, and diversity of family history. Selection bias could lead to differences in findings if other factors related to seroprevalence were related to the sampling of either cases or controls: for example, the possibility that characteristics such as stress or illness might be related to both EBV antibody status and nonparticipation in controls. However, this type of bias could exist in any study relying on volunteer participation.

Another source of selection bias could arise from selection of controls with valid driver's licenses and phone numbers, if, for example, having a driver's license were related to EBV antibody prevalence. Exclusion of the 10% of cases without driver's licenses in post-hoc analyses, however, did not notably change the findings of an association of SLE with EBV antibodies. Confounding bias is less likely to have contributed to the differences in findings. We controlled for potential confounders (e.g., age, season, and education), but found none that had an impact on the observed associations (or lack thereof). Although residual confounding or confounding by other factors (e.g., pollution or other noncausal factors) is possible, we did not see evidence of confounding by other factors such as smoking, occupation sunlight or silica exposure, or living or working on a farm (results not shown). We are unaware of other factors that could be independently and strongly associated with both EBV reactivation and SLE.

The case–control study design cannot discriminate between a role for EBV as a cause and a role for EBV as an effect of SLE pathology or treatment. In the present study serum samples were obtained a median of 13 months after diagnosis. However, the observed association with EBV-IgA antibodies did not appear to differ over time since diagnosis. We did not have information on the use of immunosuppressive medications that could affect the emergence of latent viral infection at the time blood specimens were obtained. To indirectly examine this possibility, we considered whether EBV antibodies were associated with proteinuria, a marker of renal involvement often requiring aggressive immunosuppressive or cytotoxic therapy. Unexpectedly, EBV-IgA antibodies were inversely associated with proteinuria, suggesting that the overall observed associations were not related to treatment for more severe disease.

Longitudinal data may help in the understanding of the temporal relationship between EBV seroprevalence and SLE. However, the onset of autoimmunity can occur years prior to diagnosis, and it is unclear at what

point EBV might act on the pathway spanning the initiation or promotion of disease. A recent analysis of stored serum samples in a large population of subjects from the armed forces revealed that EBV-IgG antibodies were elevated in individuals who later developed lupus (29), indicating that the association preceded clinical onset and diagnosis. Antibodies to EBV measured several years prior to disease onset have also been associated with the development of multiple sclerosis (30), raising the possibility that similar processes of immune dysregulation may occur in the development of these two very different autoimmune diseases.

The present findings might be explained by increased rates of either reinfection or reactivation of EBV, and the two scenarios may be difficult to separate. Given the high frequency of infection in the general population and the occasional loss of latency in healthy individuals, it seems likely that opportunities for reinfection may be common. Coinfection with multiple EBV genotypes has been described (31–33), suggesting that infection with one EBV genotype may not protect against secondary infection with a different strain. Although we saw no association of SLE with EBV-IgM antibodies, it is possible that reinfection from exogenous sources of EBV could play a role in the development of SLE. Alternatively, changes in EBV antibody titers may reflect a loss in the effectiveness of the immune response in controlling existing latent EBV infection, resulting in productive cycles of viral replication and reinfection of susceptible cells within an individual. The differences that we observed in the association of SLE with EBV-IgA antibodies by race, age, and CTLA-4 genotype may offer clues to the mechanisms underlying the EBV–SLE association.

The racial difference in the association between EBV-IgA and SLE is intriguing, especially since African Americans have a higher risk of SLE, tend to develop the disease earlier, and often have a more severe course of disease. In this study, African Americans were more likely than whites to have a history of EBV infection, reflected by the increased EBV-IgG seroprevalence among both African American cases and African American controls. In post-hoc analyses examining the determinants of EBV seroprevalence in controls (results not shown), African American race was the only factor associated with EBV-IgA antibodies. One explanation for the racial difference in EBV-IgA seroprevalence could be that there are more opportunities for reinfection among African Americans, given the higher population prevalence of infection and likelihood of encountering and becoming infected with new viral strains. Alternatively, other factors independently related to the immune response to EBV and loss of latency could vary by race, with the same difference being related to the differential risk of SLE. For example, certain types of stress may be related to reactivation of latent EBV infection, leading to increased viral shedding and production of EBV-IgA antibodies (26,34). Stress has also been linked to flares of SLE disease activity (although not with the onset of disease) (35). Thus, stress-related reactivation of EBV might be related to the immune activation of B cells and production of autoantibodies in SLE, and differences in the degree or type of stress experienced by African Americans could contribute to both loss of latency of EBV and increased risk of SLE.

The association between EBV-IgA and SLE was significantly stronger with increasing age in whites, and appeared stronger in older compared with younger African Americans (e.g., age >50 years versus <35 years). Cutoff points roughly based on 15-year age groups were used in stratified analyses to derive ORs comparable with those in previous analyses. However, the findings of this analysis were relatively insensitive to changes in cutoff points, and the interaction of age and EBV-IgA antibodies with respect to SLE in whites was significant in a model including age as a continuous variable. There is serologic evidence (including EBV-IgA antibodies to VCA) suggesting higher EBV reactivation rates in older individuals, which is likely due to the decrease in cell-mediated immunity seen with aging (36). One possibility, therefore, is that the effects of decreased cell-mediated immunity and loss of EBV latency may play a role in the development of SLE in some older individuals.

One proposed mechanism explaining the association of EBV and SLE is that lupus patients have altered T cell–mediated suppression of EBV-induced B cell proliferation. One recent study reported that increased EBV viral loads in SLE patients compared with controls were correlated with EBV-specific T cell responses (37). Clinical disease related to reactivation of latent EBV infections is well recognized in immunosuppressed patients, and is sometimes considered an opportunistic infection secondary to T cell immunodeficiency (38). We found that variation in the promoter region of CTLA-4, an important regulator of T cell response, significantly modified the association of SLE and EBV-IgA ($P = 0.03$), with stronger associations seen in both African Americans and whites with the CTLA-4 -1661AA genotype. There is no evidence pertaining to this polymorphism and functional differences in CTLA-4 expression; however, the presence of the -1661G allele was

associated with SLE in younger African Americans in our previous analyses (24) and with type 1 diabetes in a study of North Africans (39).

The observed differences in the SLE–EBV association by CTLA-4 genotype could be due to linkage disequilibrium with another functional locus in different parts of the CTLA-4 gene. A recent study, for instance, has described variation in the 3′-untranslated region of the CTLA-4 gene related to the expression of soluble CTLA-4 (40) and to organ-specific autoimmune diseases. Differences in soluble CTLA-4 have been seen in SLE patients (41), but it is unclear how it might be related to EBV infection. Variation in adjacent genes (i.e., ICOS, CD28) might also play a role. In an experimental study of a virus-associated autoimmune disease, Theiler's murine encephalomyelitis virus, blockade of the CD28–B7 costimulatory pathway suppressed virus-specific immune responses and exacerbated disease (42). Therapeutic manipulation of this costimulatory pathway in SLE (43), which could affect both the autoimmune response and control of viral latency, could be problematic if the progression of SLE is associated with EBV infection.

It remains unclear whether the positive results seen for EBV-IgA seroprevalence (and not overall IgG seroprevalence) are a reflection of increased sensitivity of the assay or other factors that could be specific to the generation of IgA antibodies. In healthy individuals, the majority of EBV genomes are found in IgA-positive B cells (44). It may be that characteristics of initial infection or frequency of reactivation in SLE patients could be related to the proportion of EBV-infected B cells and production of EBV-specific IgA antibodies in SLE patients. Alternatively, the presence of IgA antibodies, similar to the finding of higher IgG titers, may simply be a marker of increased viral replication in SLE patients, reflecting the increased production of capsid antigen during the active lytic phase of infection. In addition to studies showing increased EBV-IgA VCA antibodies related to stress (26,34) and aging (36), both representing states of decreased cellular immunity, increased EBV-IgA titers have been associated with human immunodeficiency virus (HIV) infection and progression of HIV disease (45,46). Considered in this context, the findings of an association between EBV-IgA antibodies and SLE seem consistent with a process of defective cell-mediated immunity related to the control of EBV infection in SLE patients.

The biology of EBV infection has yielded several hypotheses on the mechanisms that might causally link EBV to the etiology and pathology of SLE (2). One possibility is that EBV infection may alter the balance of B cell cytokine production and contribute to the pathogenesis of SLE, for example, through coding a viral homolog of IL-10 and inducing expression of human IL-10 (47,48). Variation in cytokine genes has also been associated both with the presence of EBV antibodies (10–12) and with SLE (13,14). We observed no effect modification of the EBV–SLE association by the IL-1 and TNF polymorphisms that were examined. Given that allelic variation in IL-10 has also been associated with EBV seroprevalence in healthy individuals (11,12), it would be of interest to investigate the role of IL-10 polymorphisms with respect to EBV serology in SLE.

The present study thus provides new evidence on the relationship between SLE and EBV seroprevalence, showing a strong association of EBV-IgA antibodies with SLE in African Americans and older whites compared with population-based controls. The lack of similar associations between SLE and HSV-1 or CMV antibodies suggests that the association with EBV was not due to a general immunosuppression and loss of control of latent infections. This is the first study to describe a possible relationship between CTLA-4 genotype and the patterns of EBV immune responsiveness among SLE patients. Effect modification of the SLE–EBV association by allelic variation in CTLA-4 is biologically plausible and suggests that there may be a physiologic difference among SLE patients in immune responsiveness to EBV infection. The observation of effect modification by age, race, and CTLA-4 genotype should be examined in other studies and may help frame additional hypotheses on the role of EBV in SLE etiology.

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