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Epstein-Barr Virus Serologic Abnormalities and risk of Rheumatoid Arthritis among Women

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Abstract

Background—EBV infection and the immune response may be involved in the pathogenesis of rheumatoid arthritis (RA). Past studies have suggested an association between EBV and RA.

Methods—We studied the association between EBV serologies and RA risk in a nested case-control study in the Nurses' Health Study cohorts. We confirmed incident RA cases from 1990–2002 by questionnaire and medical record review. Each incident case with blood collected prior to RA symptoms was matched to a healthy participant by time of day and date of blood collection, birth year, menopausal status and postmenopausal hormone use. Immunofluorescence assays measured serologic EBV responses: viral capsid antigen (VCA), early-antigen-diffuse (EA-D) and early antigen-complex (EA-restricted and diffuse), Epstein Barr nuclear antigen (EBNA)-1, EBNA-2 and cytomegalovirus (CMV), as control. All were reported as titers, except BZLF-1 and CMV, which were reported as positive or negative. ANA positive samples were excluded. Elevated EBV antibody titers were defined as the upper 20% (or nearest titer) among controls. Conditional logistic regression analyses modeled RA risk associated with elevated EBV titers or the presence/absence CMV, further adjusted for pack-years smoking and alcohol intake.

Results—87 incident RA cases were identified. Mean time to RA after blood draw was 6.2 (±3.5) years in NHS and 1.9 (±0.6) years in NHSII. Antibody titers against EBV were not significantly different between pre-RA cases and controls.

Conclusions—In this prospective study of women, we observed no association between EBV serologies and RA risk.

Keywords

rheumatoid arthritis; Epstein Barr virus; virus; risk factors; epidemiology

RA is an autoimmune disease characterized by chronic, destructive and debilitating arthritis, occasionally with systemic involvement. RA is the most common inflammatory arthritis, affecting approximately 1% of the population(1). The etiology of RA is unknown, but it is presumed to have a genetic basis, with environmental factors contributing to its development. A viral trigger for RA in the genetically-predisposed has been hypothesized

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Competing Interests
None of the authors have any competing interests to declare.

for many years(2–5), and among the many environmental agents which could be involved in RA pathogenesis, Epstein-Barr virus (EBV) has generated considerable interest. EBV, the agent of infectious mononucleosis, is a highly prevalent DNA-containing herpes virus, infecting > 98% of humans by age 40(6).

There are many reasons to hypothesize that EBV infection, or the immune response it engenders, may be related to the development of RA. EBV was first implicated in RA by Alspaugh and Tan, who reported that sera from patients with RA was reactive against an antigen in EBV-transformed lymphocytes(4, 7). This “RA nuclear antigen” was a glycine/alanine rich repeat in Epstein-Barr nuclear antigen-1 (EBNA-1)(8, 9). Antibodies against this repeat cross-react with a 62kD protein in RA, but not normal, synovium(10–12). Sequence similarities exist between other EBV and RA-specific proteins. The EBV protein gp110 has homology with the QKRAA amino acid motif of the HLA-SE(13, 14) and the EBNA-1 p107 peptide cross-reacts with collagen and keratin(15).

We aimed to test the hypothesis that EBV is associated with risk of developing RA by investigating anti-EBV serologies prior to RA onset, employing a prospective cohort study with data and blood samples collected years in advance of RA onset. Anti-cytomegalovirus (CMV) antibody titers were chosen as a comparison because CMV is a herpes virus that, like EBV, is ubiquitous in humans. The evidence linking CMV to RA is not as strong as that for EBV(16–18), and elevated anti-CMV antibody titers have not been linked with other autoimmune diseases(19, 20) or SLE(21–23).

Methods

Study Population

The NHS is a prospective cohort of 121,700 female nurses, aged 30–55 years in 1976, when the study began. The NHSII was established in 1989, when 116,608 female nurses aged 25–42 completed a baseline questionnaire about their medical histories and lifestyles. Ninety-four percent of the NHS participants from 1976–2002 and 95% of NHSII participants from 1989–2003 have remained in active follow-up (5–6% no longer respond to questionnaires and have not been confirmed as dead). All aspects of this study were approved by the Partners’ HealthCare Institutional Review Board.

Identification of RA

As previously described(24), we employed a two-stage procedure in which all nurses who self-reported any connective tissue disease received a screening questionnaire for connective tissue disease symptoms(25), and, if positive, a detailed medical record review for American College of Rheumatology (ACR) classification criteria for RA(26), in order to identify and validate incident cases of RA. The presence or absence of rheumatoid factor and other features of RA were based on medical record review. Subjects with four of the seven ACR criteria documented in the medical record were considered to have definite RA.

Population for Analysis

We excluded prevalent RA cases diagnosed before the start of the cohort, non-responders, and women who reported any connective tissue disease that was not subsequently confirmed to be RA by medical record review. Women were censored when they failed to respond to any subsequent biennial questionnaires. Among the women in each cohort who had provided a sample for serologic analyses, each participant with confirmed incident RA after the blood sample was collected and matched by year of birth, menopausal status, and postmenopausal hormone use to a healthy woman in the same cohort without RA.

Blood sampling

From 1989 to 1990, 32,826 (27%) NHS participants ages 43 to 70 agreed to provide blood samples for future NHS studies. Between 1996 and 1999, 29,613 (25%) of the women participating in NHSII cohort, ages 32–52 at that time, also agreed to have their blood drawn for future investigations. All samples were collected in heparinized tubes and sent to us by overnight courier in chilled containers. On receipt, the blood samples were centrifuged, aliquoted, and stored in liquid nitrogen freezers at −70° F.

EBV and CMV Serologic Studies

Virologic assays were performed at the Swedish Institute for Infectious Disease Control (Dr. Kerstin Falk, Director, Stockholm, Sweden), as in past NHS studies investigating their association with risk of MS(19). We measured IgG antibodies to EBV viral capsid antigen(VCA), early antigen- diffuse (EA-D) and early antigen complex (EA-complex) by indirect immunofluorescence (IF). IgG antibodies against EBNA-1 and EBNA-2 were measured by anti-complement IF(27). Unlike ELISA methods for which there are different antigen preparations, lack of standardization and more variability, IF assays are well-standardized and more sensitive(6, 28–30). Samples were first inactivated for 30 minutes at 56 degrees C. Negative control cells were added to the samples at the lowest dilution to detect non-specific staining and detection of antinuclear antibodies by IF was also reported. Anti-CMV IgG antibody titers were performed by indirect IF as a control. Titers of antibodies to EBV and CMV antigens have been shown to be stable, reproducible and informative in past studies(19, 20, 31–36).

Anti-CCP detection

Testing for anti-CCP antibodies was performed using the DIASTAT™ anti-CCP 2nd generation test, a semi-quantitative/qualitative enzyme-linked immunosorbent assay (ELISA) for detection of IgG anti-CCP antibodies. Anti-CCP antibody titer >5 U/ml was considered positive. Anti-CCP titers measured on 5 splits of each of 6 QC specimens yielded perfect reproducibility.

Validation Substudies

Prior to the start of these investigations, we performed three pilot validation studies in the lab of Dr. Falk in Sweden. In the first pilot, 28 plasma NHS samples were tested in blinded splits for EBNA-1, EBNA-2, VCA, EA-D, EA-complex, and CMV. Most results were a perfect match for all antigens and no results differed by > 1 dilution. In the second pilot, we verified that a delay in processing blood samples (at 0, 24, or 48 hours after receipt) did not affect titers in 49 heparinized plasma samples. Most results were a perfect match and no results were off > 2 dilutions. In the third pilot study, we addressed the issue of potential cross-reactivity with RF. IgM RF may cross-react in serologic tests, in particular in IgM assays. The EBV IF assays employed here however detected IgG, not IgM. There is historical concern that RF may cross-react in EBV serologic assays, but such interference has not been detected in most studies(37, 38). Four RF+ samples were run over a sepharose column without IgG, or over an affinity chromatography Human IgG Sepharose 6 Fast Flow column™ (39)(GE Healthcare Biosciences, Piscataway, NJ) to deplete RF. The samples were sent to the Falk lab as blinded splits. We confirmed that RF was completely depleted and observed no effect of RF, or anti-CCP antibody titers by ELISA (40),on anti-EBV titers. Most results were a perfect match and no results were off > 2 dilutions.

HLA-DRB1 shared epitope (*HLA-SE*) determination

Low resolution HLA-DRB1 genotyping was performed in both cases and controls using polymerase chain reaction with sequence specific primers (PCR-SSP) using OLERUP SSP

kits (QIAGEN, West Chester, PA). We used primers to amplify DNA samples that contained sequences for HLA-DRB1*04, *01,*10 and *14, along with consensus primers and appropriate positive and negative control samples. For samples with positive signals 2-digit HLA signals, sequence specific primers were used for high resolution 4-digit shared epitope allele detection of DRB1*0401, *0404, *0405, *0101, *0102, *1402, and *1001. OLERUP SSP computer software (QIAGEN, West Chester PA) was used to determine 4-digit HLA types. Quality control split samples were included randomly interspersed with study samples.

Covariate information

Subjects in both cohorts have completed biennial questionnaires regarding diseases, lifestyle, and health practices. Age was updated in each cycle. Age at menarche was reported on the baseline questionnaire in both cohorts. Data on parity, menopausal status and postmenopausal hormone use were selected from the questionnaire cycle prior to the date of RA diagnosis (or index date in controls). Self-reported menopausal status and age at menopause are highly reproducible in our cohorts: in a validation study of a subsample of NHS participants, 82% of naturally postmenopausal women reported the same age at menopause (within one year) on two questionnaires mailed two years apart(41). Participants in both cohorts were asked at baseline whether they were a current smoker or had ever smoked in the past and the age at which they began to smoke. Current smokers were asked for the number of cigarettes typically smoked per day and former smokers reported the age at which they stopped smoking and the number of cigarettes smoked per day before quitting. On each subsequent questionnaire, participants reported whether they currently smoked and the number of cigarettes smoked per day. From these reports, we calculated pack years of smoking (product of years of smoking and packs of cigarettes per day). Other potential confounders examined included body mass index (BMI), which was computed for each two-year time interval using the most recent weight in kilograms divided by height in meters squared as reported at baseline. Alcohol intake was reported at least every four years and categorized in grams/day.

Statistical Analysis

We calculated the clinical characteristics of the RA cases at diagnosis in both cohorts, as well as the demographic characteristics of the RA cases compared to their healthy matched controls. Separate analyses were employed for the titers of each of the antibodies (VCA, EA-D, EA-complex, EBNA-1 and EBNA-2, and CMV). Elevated EBV antibody titers were defined as the upper 20% (or nearest titer) among controls(42) and EBV titers were also analyzed as geometric mean titers(GMTs), two different approaches used in the past studies of EBV in NHS(19). EBNA1:EBNA2 ratio was also calculated for each individual as low ratios (<1) have been shown to correlate with severe, resolved EBV infection (42). For each viral serology, conditional logistic regression analyses, conditioned on matching factors, were employed to model the risk of RA associated with elevated EBV titers (defined by top 20% among the controls) or the presence/absence of CMV. We assessed for confounding by further adjusting for pack years of cigarette smoking, parity and breastfeeding and body mass index, assessed prior to diagnosis of RA. We also compared viral antibody geometric mean titers (GMT) in cases and controls using *paired mean difference* tests (to account for the matching of cases and controls). Conditional logistic regression models were employed to estimate the risk of RA in cases compared to controls associated with the anti-viral antibody GMT, while controlling for other potential confounders. SAS version 9.1 (SAS Institute, Cary, NC) was used for all analyses.

Results

Ninety-three incident RA cases were identified. Six ANA-positive RA cases and their matched controls were excluded from analyses. The characteristics of the 87 RA cases at diagnosis in each of the two cohorts are shown in Table 1. Women in the NHS cohort were older at RA onset and their blood was drawn more years before the diagnosis of RA, compared to the women in the NHS2 cohort. Correspondingly, a smaller proportion on the NHS RA cases were anti-CCP antibody positive at the time of the blood draw, but the proportion of *HLA-DRB1* shared epitope positive cases was higher in NHS than NHS2. The cases in the NHS cohort also had a slightly higher proportion of RF positivity, more radiographic erosions, and rheumatoid nodules present at RA diagnosis and a slightly higher mean number of American College of Rheumatology criteria for the classification of RA, although both cohorts had a high frequency of hand arthritis.

Table 2 shows the characteristics of the RA cases and their matched controls at the time of RA diagnosis (or index date for the controls). The proportions of pre- and post-menopausal women among cases and controls were similar in each of the cohorts, as were the proportions currently receiving postmenopausal hormones. Other factors, including cigarette smoking, age at menarche and parity, alcohol intake and BMI, were also similar in the two groups.

The EBV and CMV serologic results for the RA cases and controls are displayed in Table 3. The final multivariable model included only pack years of cigarette smoking and alcohol intake. None of the serologies was statistically associated with future RA case status. Further adjustment for age at menarche, parity, and BMI did not significantly influence results. In analyses in which 4-fold elevations of each of the serologic titers were considered, we found no evidence that any were associated with increased risk of developing RA (Table 4). We did not find any evidence of effect modification by *HLA-DRB1* shared-epitope or by the presence of anti-CCP antibodies.

Discussion

In these prospective analyses utilizing stored blood samples for two large cohorts of women followed for the development of RA, we have found no relationship between EBV serologic responses and the risk of RA years later. Several past cross-sectional studies have shown elevated levels of EBV antibodies in patients with RA, and higher quantities of EBV DNA have been isolated from individuals with RA than controls(37, 38, 43–45).A synergistic effect of EBV exposure and HLA type on the risk of RA has been reported(46). RA patients have been found to have 10-fold higher EBV DNA loads in peripheral blood mononuclear cells compared to controls and this elevation was not influenced by RF, age, RA duration, or use of immunosuppressive or anti-TNF therapies(45, 47). Several studies have shown that EBV DNA and mRNA levels are much higher in RA compared to normal synovium(37, 46, 48, 49). Synovial EBV DNA loads are highest in RA patients with ≥ 1 copy of the HLA-SE(46). EBV-specific T cell function is also impaired in RA(50–56).A large proportion of CD8+ T cells infiltrating rheumatoid synovium recognize EBV transactivating factors, BZLF-1 and BMLF-1, important in control of EBV reactivation(57). It is unclear, however, whether the elevated levels of antiviral antibodies and viral DNA found in RA patients are present prior to RA onset, indicating viral involvement in the pathogenesis of RA, or rise with RA onset, due to the dysregulated immunity of RA.

Elevated anti-EBV antibody titers have been associated prospectively with multiple sclerosis (MS) and systemic lupus erythematosus (SLE), autoimmune diseases related to RA. MS, like RA, is a T cell-dependent autoimmune disease that disproportionately affects women. In

a nested case-control study within the Nurses' Health Study, Ascherio and colleagues prospectively examined anti-EBV antibody titers in women who later developed MS, compared to those in healthy controls(19). Higher antibody titers to EBNA-1, EBNA-2 and EA-D, but not cytomegalovirus (CMV), were found in those who developed MS. A 4-fold increase in anti-EBNA-2 titer was associated with a 4-fold increase in MS risk(19). In a nested case-control study, detectable plasma EBV was associated with increased MS risk, although EBV viral load was not(58). The elevated risk of MS associated with increased anti-EBV antibody titers was confirmed in another large prospective cohort, U.S. military recruits at the Department of Defense(20). EBV serologic patterns suggest EBV is reactivated up to 20 years before clinical MS. Titers of anti-EBNA-1 and anti-VCA antibodies were significantly higher in U.S. military recruits who later developed SLE than in normal individuals, a finding not observed with CMV. Antibody titers rose gradually from their first detectable levels years prior to the first symptoms of SLE until the time of SLE diagnosis, paralleling, and in some cases preceding, development of SLE-specific antibodies(21, 22).

Despite speculation about the potential role of EBV in triggering autoimmune diseases, including RA, as well as MS and SLE, studies to date have been limited by cross-sectional or retrospective designs and there has only been one past prospective study examining anti-EBV antibodies and risk of RA(59). Jorgensen and colleagues conducted a case–control study nested in a cohort of Norwegian blood donors. They identified 49 premorbid sera from donors who later developed RA and matched them to controls. Anti-EBV antibodies were reported as positive or negative using ELISA kits. This study did not detect any increased risk of developing RA associated with anti-EBV antibodies by this method.

Our study employed anti-EBV titers by immunofluorescence assays rather than less reliable ELISA kits, and ruled out possible interference by ANA or RF positivity. Our study also was larger and with much more detailed prospectively-collected covariate data than the only other past prospective study. The single blood draw within the NHS cohort is a potentially limiting factor. The ideal study design to assess potential associations between EBV and RA development would be sequential blood samples on patients, but this is a limitation within the NHS cohort that cannot be altered. The main limitation of our current study however is the potential lack of power to detect a small effect size. With 87 cases and matched controls, we had limited power detect a weak association between EBV serologies and future RA risk, and our results can only exclude a large effect. We did not find an additive interaction association between EBV serologies and risk of RA for HLA-SE positive RA and/or anti-CCP antibody positive RA, but again this may have been due to limited statistical power. In summary, despite a wealth of past *in vitro* and cross-sectional RA population data, we did not detect a strong association between EBV serologic responses and future risk of developing RA in this cohort of women. Even larger population studies may be warranted to continue to address the potential prospective association.

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References

1. Myasoedova E, Crowson CS, Kremers HM, Therneau TM, Gabriel SE. Is the incidence of rheumatoid arthritis rising?: results from Olmsted County, Minnesota, 1955–2007. Arthritis Rheum. 62(6):1576–82. [PubMed: 20191579]
2. Albert LJ, Inman RD. Molecular mimicry and autoimmunity. N Engl J Med. 1999; 341(27):2068–74. [PubMed: 10615080]
3. Bonneville M, Scotet E, Peyrat MA, Saulquin X, Houssaint E. Epstein-Barr virus and rheumatoid arthritis. Rev Rhum Engl Ed. 1998; 65(6):365–8. [PubMed: 9670326]
4. Alspaugh MA, Jensen FC, Rabin H, Tan EM. Lymphocytes transformed by Epstein-Barr virus. Induction of nuclear antigen reactive with antibody in rheumatoid arthritis. J Exp Med. 1978; 147(4):1018–27. [PubMed: 206643]
5. Cooke SP, Rigby SP, Griffiths DJ, Venables PJ. Viral studies in rheumatic disease. Ann Med Interne (Paris). 1998; 149(1):30–3. [PubMed: 11490514]
6. Linde, A. Epstein-Barr Virus. In: Murray, PRBE.; Pfaller, MA.; Tenover, FC.; Yolken, RH., editors. Manual of Clinical Microbiology. 8. Washington, D.C: ASM press; 2003. p. 1331-40.
7. Alspaugh MA, Tan EM. Serum antibody in rheumatoid arthritis reactive with a cell-associated antigen. Demonstration by precipitation and immunofluorescence. Arthritis Rheum. 1976; 19(4): 711–9. [PubMed: 1085148]
8. Baboonian C, Halliday D, Venables PJ, Pawlowski T, Millman G, Maini RN. Antibodies in rheumatoid arthritis react specifically with the glycine alanine repeat sequence of Epstein-Barr nuclear antigen-1. Rheumatol Int. 1989; 9(3–5):161–6. [PubMed: 2481875]
9. Petersen J, Rhodes G, Roudier J, Vaughan JH. Altered immune response to glycine-rich sequences of Epstein-Barr nuclear antigen-1 in patients with rheumatoid arthritis and systemic lupus erythematosus. Arthritis Rheum. 1990; 33(7):993–1000. [PubMed: 2164400]
10. Rumpold H, Rhodes GH, Bloch PL, Carson DA, Vaughan JH. The glycine-alanine repeating region is the major epitope of the Epstein-Barr nuclear antigen-1 (EBNA-1). J Immunol. 1987; 138(2):593–9. [PubMed: 2432130]
11. Kouri T, Petersen J, Rhodes G, Aho K, Palosuo T, Heliovaara M, et al. Antibodies to synthetic peptides from Epstein-Barr nuclear antigen-1 in sera of patients with early rheumatoid arthritis and in preillness sera. J Rheumatol. 1990; 17(11):1442–9. [PubMed: 2177109]
12. Fox R, Sportsman R, Rhodes G, Luka J, Pearson G, Vaughan J. Rheumatoid arthritis synovial membrane contains a 62,000-molecular-weight protein that shares an antigenic epitope with the Epstein-Barr virus-encoded associated nuclear antigen. J Clin Invest. 1986; 77(5):1539–47. [PubMed: 2422209]
13. Roudier J, Petersen J, Rhodes GH, Luka J, Carson DA. Susceptibility to rheumatoid arthritis maps to a T-cell epitope shared by the HLA-Dw4 DR beta-1 chain and the Epstein-Barr virus glycoprotein gp110. Proc Natl Acad Sci U S A. 1989; 86(13):5104–8. [PubMed: 2472638]
14. Lotz M, Roudier J. Epstein-Barr virus and rheumatoid arthritis: cellular and molecular aspects. Rheumatol Int. 1989; 9(3–5):147–52. [PubMed: 2481874]
15. Birkenfeld P, Haratz N, Klein GDS. Cross-reactivity between EBNA 1p107 peptide, collagen and keratin: implications for the pathogenesis of rheumatoid arthritis. Clin Immunol Immunopathol. 1989; 54:14–25. [PubMed: 1688406]
16. Inman RD. Infectious etiology of rheumatoid arthritis. Rheum Dis Clin North Am. 1991; 17(4): 859–70. [PubMed: 1662820]
17. Carty SM, Snowden N, Silman AJ. Should infection still be considered as the most likely triggering factor for rheumatoid arthritis? J Rheumatol. 2003; 30(3):425–9. [PubMed: 12610794]
18. Balandraud N, Roudier J, Roudier C. Epstein-Barr virus and rheumatoid arthritis. Autoimmun Rev. 2004; 3(5):362–7. [PubMed: 15288002]
19. Ascherio A, Munger KL, Lennette ET, Spiegelman D, Hernan MA, Olek MJ, et al. Epstein-Barr virus antibodies and risk of multiple sclerosis: a prospective study. Jama. 2001; 286(24):3083–8. [PubMed: 11754673]

20. Levin LI, Munger KL, Rubertone MV, Peck CA, Lennette ET, Spiegelman D, et al. Temporal relationship between elevation of epstein-barr virus antibody titers and initial onset of neurological symptoms in multiple sclerosis. Jama. 2005; 293(20):2496–500. [PubMed: 15914750]
21. McClain MT, Bruner TL, Dennis GJ, Harley JB, James JA. The temporal relationship between the onset of anti-EBNA-1 and lupus atuoimmunity supports a role for EBV in the development if SLE [abstract]. Arthritis Rheum. 2003; 48(9 suppl):S674.
22. Heinlen LD, McClain MT, Dennis GJ, Rubertone MV, Harley JB, James JA. The development of antibodies targeting Epstein-Barr virus closely parallels autoimmune progression near the onset of SLE [abstract]. Arthritis Rheum. 2003; 48(9 suppl):S662.
23. Parks CG, Cooper GS, Hudson LL, Dooley MA, Treadwell EL, St Clair EW, et al. Association of Epstein-Barr virus with systemic lupus erythematosus: Effect modification by race, age, and cytotoxic T lymphocyte-associated antigen 4 genotype. Arthritis Rheum. 2005; 52(4):1148–59. [PubMed: 15818712]
24. Costenbader KH, Feskanich D, Mandl LA, Karlson EW. Smoking intensity, duration, and cessation, and the risk of rheumatoid arthritis in women. Am J Med. 2006; 119(6):503, e1–9. [PubMed: 16750964]
25. Karlson EW, Sanchez-Guerrero J, Wright EA, Lew RA, Daltroy LH, Katz JN, et al. A connective tissue disease screening questionnaire for population studies. Ann Epidemiol. 1995; 5(4):297–302. [PubMed: 8520712]
26. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum. 1988; 31(3):315–324. [PubMed: 3358796]
27. Lennette ET, Rymo L, Yadav M, Masucci G, Merk K, Timar L, et al. Disease-related differences in antibody patterns against EBV-encoded nuclear antigens EBNA 1, EBNA 2 and EBNA 6. Eur J Cancer. 1993; 29A(11):1584–9. [PubMed: 8217366]
28. Svahn A, Magnusson M, Jagdahl L, Schloss L, Kahlmeter G, Linde A. Evaluation of three commercial enzyme-linked immunosorbent assays and two latex agglutination assays for diagnosis of primary Epstein-Barr virus infection. J Clin Microbiol. 1997; 35(11):2728–32. [PubMed: 9350722]
29. Bruu AL, Hjetland R, Holter E, Mortensen L, Natas O, Petterson W, et al. Evaluation of 12 commercial tests for detection of Epstein-Barr virus-specific and heterophile antibodies. Clin Diagn Lab Immunol. 2000; 7(3):451–6. [PubMed: 10799460]
30. Gartner BC, Fischinger JM, Roemer K, Mak M, Fleurent B, Mueller-Lantzsch N. Evaluation of a recombinant line blot for diagnosis of Epstein-Barr Virus compared with ELISA, using immunofluorescence as reference method. J Virol Methods. 2001; 93(1–2):89–96. [PubMed: 11311347]
31. Sidorchuk A, Lagarde F, Pershagen G, Wickman M, Linde A. Epstein-Barr virus infection is not associated with development of allergy in children. Pediatr Infect Dis J. 2003; 22(7):642–7. [PubMed: 12867841]
32. 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):109–21. [PubMed: 3029317]
33. Nordstrom M, Hardell L, Linde A, Schloss L, Nasman A. Elevated antibody levels to Epstein-Barr virus antigens in patients with hairy cell leukemia compared to controls in relation to exposure to pesticides, organic solvents, animals, and exhausts. Oncol Res. 1999; 11(11–12):539–44. [PubMed: 10905566]
34. Nordstrom M, Hardell L, Lindstrom G, Wingfors H, Hardell K, Linde A. Concentrations of organochlorines related to titers to Epstein-Barr virus early antigen IgG as risk factors for hairy cell leukemia. Environ Health Perspect. 2000; 108(5):441–5. [PubMed: 10811571]
35. Hardell E, Eriksson M, Lindstrom G, Van Bavel B, Linde A, Carlberg M, 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):619–29. [PubMed: 11697490]

36. Akre O, Lipworth L, Tretli S, Linde A, Engstrand L, Adami HO, et al. Epstein-Barr virus and cytomegalovirus in relation to testicular-cancer risk: a nested case-control study. Int J Cancer. 1999; 82(1):1–5. [PubMed: 10360811]
37. Blaschke S, Schwarz G, Moneke D, Binder L, Muller G, Reuss-Borst M. Epstein-Barr virus infection in peripheral blood mononuclear cells, synovial fluid cells, and synovial membranes of patients with rheumatoid arthritis. J Rheumatol. 2000; 27(4):866–73. [PubMed: 10782808]
38. Catalano MA, Carson DA, Slovin SF, Richman DD, Vaughan JH. Antibodies to Epstein-Barr virus-determined antigens in normal subjects and in patients with seropositive rheumatoid arthritis. Proc Natl Acad Sci U S A. 1979; 76(11):5825–8. [PubMed: 230491]
39. Matsumoto A, Kojima N, Takeuchi F, Mizuochi T. Kinetic analysis of interaction of different types of rheumatoid factors with immobilized IgG using surface plasmon resonance. J Biochem (Tokyo). 2000; 128(6):1009–16. [PubMed: 11098144]
40. Lee, D.; Schur, P. personal communication.
41. Colditz GA, Stampfer MJ, Willett WC, Stason WB, Rosner B, Hennekens CH, et al. Reproducibility and validity of self-reported menopausal status in a prospective cohort study. Am J Epidemiol. 1987; 126(2):319–325. [PubMed: 3605058]
42. Bertrand KA, Birmann BM, Chang ET, Spiegelman D, Aster JC, Zhang SM, et al. A prospective study of Epstein-Barr virus antibodies and risk of non-Hodgkin lymphoma. Blood. 2010; 116(18): 3547–53. [PubMed: 20647565]
43. Ferrell PB, Aitcheson CT, Pearson GR, Tan EM. Seroepidemiological study of relationships between Epstein-Barr virus and rheumatoid arthritis. J Clin Invest. 1981; 67(3):681–7. [PubMed: 6259207]
44. Hazelton RA, Sculley TB, Pope JH. The prevalence of antibodies to an Epstein-Barr virus-induced polypeptide (EBNA-2) in the sera of rheumatoid arthritic families. Br J Rheumatol. 1987; 26(3): 193–6. [PubMed: 3034370]
45. Balandraud N, Meynard JB, Auger I, Sovran H, Mugnier B, Reviron D, et al. Epstein-Barr virus load in the peripheral blood of patients with rheumatoid arthritis: accurate quantification using real-time polymerase chain reaction. Arthritis Rheum. 2003; 48(5):1223–8. [PubMed: 12746895]
46. Saal JG, Krimmel M, Steidle M, Gerneth F, Wagner S, Fritz P, et al. Synovial Epstein-Barr virus infection increases the risk of rheumatoid arthritis in individuals with the shared HLA-DR4 epitope. Arthritis Rheum. 1999; 42(7):1485–96. [PubMed: 10403278]
47. Balandraud N, Guis S, Baptiste Meynard J, Auger I, Roudier J, Roudier C. Long-term treatment with methotrexate or tumor necrosis factor alpha inhibitors does not increase epstein-barr virus load in patients with rheumatoid arthritis. Arthritis Rheum. 2007; 57(5):762–7. [PubMed: 17530675]
48. Takei M, Mitamura K, Fujiwara S, Horie T, Ryu J, Osaka S, et al. Detection of Epstein-Barr virus-encoded small RNA 1 and latent membrane protein 1 in synovial lining cells from rheumatoid arthritis patients. Int Immunol. 1997; 9(5):739–43. [PubMed: 9184919]
49. Takeda T, Mizugaki Y, Matsubara L, Imai S, Koike T, Takada K. Lytic Epstein-Barr virus infection in the synovial tissue of patients with rheumatoid arthritis. Arthritis Rheum. 2000; 43(6): 1218–25. [PubMed: 10857780]
50. Bardwick PA, Bluestein HG, Zvaifler NJ, Depper JM, Seegmiller JE. Altered regulation of Epstein-Barr virus induced lymphoblast proliferation in rheumatoid arthritis lymphoid cells. Arthritis Rheum. 1980; 23(6):626–32. [PubMed: 6248075]
51. Depper JM, Bluestein HG, Zvaifler NJ. Impaired regulation of Epstein-Barr virus-induced lymphocyte proliferation in rheumatoid arthritis is due to a T cell defect. J Immunol. 1981; 127(5): 1899–902. [PubMed: 6271870]
52. Tosato G, Steinberg AD, Blaese RM. Defective EBV-specific suppressor T-cell function in rheumatoid arthritis. N Engl J Med. 1981; 305(21):1238–43. [PubMed: 6270556]
53. Gaston JS, Rickinson AB, Epstein MA. Epstein-Barr virus-specific cytotoxic T cell responses in rheumatoid arthritis patients. Rheumatol Int. 1982; 2(4):155–9. [PubMed: 6302817]
54. Stierle HE, Brown KA, Perry JD, Holborow EJ. Increased responsiveness of rheumatoid B lymphocytes to stimulation by Epstein-Barr virus. Rheumatol Int. 1983; 3(1):7–11. [PubMed: 6310725]

55. Kahan A, Amor B, Menkes CJ. Different defects of T cell regulation of Epstein-Barr virus-induced B cell activation in rheumatoid arthritis. Arthritis Rheum. 1985; 28(9):961–70. [PubMed: 2994687]

56. Toussirot E, Wendling D, Tiberghien P, Luka J, Roudier J. Decreased T cell precursor frequencies to Epstein-Barr virus glycoprotein Gp110 in peripheral blood correlate with disease activity and severity in patients with rheumatoid arthritis. Ann Rheum Dis. 2000; 59(7):533–8. [PubMed: 10873963]

57. Scotet E, David-Ameline J, Peyrat MA, Moreau-Aubry A, Pinczon D, Lim A, et al. T cell response to Epstein-Barr virus transactivators in chronic rheumatoid arthritis. J Exp Med. 1996; 184(5): 1791–800. [PubMed: 8920867]

58. Wagner HJ, Munger KL, Ascherio A. Plasma viral load of Epstein-Barr virus and risk of multiple sclerosis. Eur J Neurol. 2004; 11(12):833–4. [PubMed: 15667414]

59. Jorgensen KT, Wiik A, Pedersen M, Hedegaard CJ, Vestergaard BF, Gislefoss RE, et al. Cytokines, autoantibodies and viral antibodies in premorbid and postdiagnostic sera from patients with rheumatoid arthritis: case-control study nested in a cohort of Norwegian blood donors. Ann Rheum Dis. 2008; 67(6):860–6. [PubMed: 17644543]

Table 1

Characteristics of the Pre-RA cases in the Nurses' Health Study (NHS) and Nurses' Health Study II (NHS2) Cohorts

	NHS N=76	NHSII N=11
Time from blood draw until RA diagnosis, years (± SD)	6.2 (3.5)	1.9 (0.6)
Anti-CCP antibody positive at blood draw, (%)	21 (27.6)	4 (36.4)
***HLA-DRB1* shared-epitope positive*, (%)**	54 (71.1)	6 (54.5)
Age at diagnosis, years (±SD)	62.6 (8.2)	46.0 (5.5)
Number of ACR criteria at diagnosis, (±SD)	4.5 (0.8)	4.2 (0.6)
Rheumatoid factor present at RA diagnosis, (%)	42 (55.3)	5 (45.5)
Radiographic erosions present at RA diagnosis, (%)	17 (22.4)	2 (18.2)
Rheumatoid nodules present at RA diagnosis, (%)	10 (13.2)	0 (0)
Hand arthritis at diagnosis, (%)	75 (99)	11 (100)

* one or more shared-epitope copies

Table 2

Characteristics of Pre-RA Cases and Matched Healthy Women within a Nested Case-Control Study in the Nurses' Health Study Cohorts

	Pre-RA Cases (n=87)	Controls (n=87)	
Matching factors at blood draw			
Age, mean (±SD)	54.9 (8.3)	54.8 (±8.2)	
Postmenopausal, (%)	55 (63)	55 (63)	
Postmenopausal hormone use at blood draw, (%)	33 (60)	33 (60)	
Other Characteristics			**p** *
Caucasian, (%)	87 (100.0)	85 (97.7)	0.50
Ever cigarette smokers, (%)	48 (55.2)	50 (57.5)	0.87
Pack-years among smokers, mean (±SD)	19.9 (14.6)	23.3 (21.1)	0.84
Age at menarche < 12 years, (%)	16 (18.6)	16 (18.4)	1.0
Parous, (%)	83 (95.4)	82 (95.4)	0.75
Alcohol intake in gms/day, mean (±SD)	3.6 (3.4)	5.0 (5.6)	0.94
Body mass index in kg/m^2, mean (±SD)	26.2 (4.8)	26.1 (5.1)	0.62

* p value from Fisher's exact tests for categorical variables and t-tests for continuous variables.

Table 3

Association of EBV Serologies by Titer Cut-offs with Risk of RA

Anti-EBV Titer	Pre-RA Cases	Matched Controls	Univariable Odds Ratio[1] (95% CI)	Multivariable Odds Ratio[2] (95% CI)
Anti-EBNA1				
<1: 80	56	61	1.0 ref.	1.0 ref.
>=1: 80	31	26	1. 29 (0.69, 2.44)	1.29 (0.66, 2.47)
Anti-EBNA2				
<1: 80	66	68	1.0 ref.	1.0 ref.
>=1: 80	21	19	1.13 (0.57, 2.27)	1.28 (0.62, 2.64)
Anti-EBNA1: Anti-EBNA2 ratio				
≤ 1.0	19	18	1.0 (ref.)	1.0 (ref.)
> 1.0	68	69	0.92 (0.41, 2.08)	0.86 (0.37, 1.97)
Anti-VCA				
<1: 5120	72	70	1.0 ref.	1.0 ref.
>= 1: 5120	15	17	0.82 (0.34, 1.97)	0.60 (0.23, 1.57)
Anti-EA Diffuse				
<1:20	80	84	1.0 ref.	1.0 ref.
>= 1:20	7	3	3.00 (0.61,14.86)	3.25 (0.64, 16.51)
Anti-EA Complex				
< 1:20	71	68	1.0 ref.	1.0 ref.
>=1: 20	16	19	0.80 (0.37, 1.71)	0.88 (0.39, 1.99)
Anti-CMV				
Negative	22	34	1.0 ref.	1.0 ref.
Positive	65	53	1.86 (0.97, 3.56)	1.77 (0.89, 3.54)

EBNA: Epstein Barr nuclear antigen, VCA: viral capsid antigen, EA: early antigen, CMV: cytomegalovirus; CI: confidence interval

[1]Univariable conditional logistic regression, conditioned on matching factors

[2]Multivariable conditional logistic regression, conditioned on matching factors, and adjusting for packyears of smoking and alcohol intake.

Table 4

The Odds of Developing RA associated with a 4-fold elevation in each EBV serology

Serology	Conditional Logistic Regression Model	OR (95%CI)
Anti-EBNA-1	Unadjusted	1.04 (0.76,1.44)
	Adjusted for smoking and alcohol	1.06 (0.75, 1.48)
Anti-EBNA-2	Unadjusted	1.06 (0.79, 1.41)
	Adjusted for smoking and alcohol	1.08 (0.79, 1.47)
Anti-VCA	Unadjusted	1.03 (0.76, 1.39)
	Adjusted for smoking and alcohol	1.01 (0.74, 1.37)
Anti-EA-Diffuse	Unadjusted	7.16 (0.53, 96.19)
	Adjusted for smoking and alcohol	8.23 (0.58, 117.72)
Anti-EA-Complex	Unadjusted	0.95 (0.67, 1.36)
	Adjusted for smoking and alcohol	1.03 (0.70,1.51)

EBNA: Epstein Barr nuclear antigen, VCA: viral capsid antigen, EA: early antigen; CI: confidence interval

[1]Univariable conditional logistic regression, conditioned on matching factors

[2]Multivariable conditional logistic regression, conditioned on matching factors, and adjusting for packyears of smoking and alcohol intake.