Antibody Responses to Epstein-Barr Virus are Altered in the Preclinical Period of Rheumatoid Arthritis

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Background

Viral infections, including infection with Epstein-Barr virus (EBV), have been suggested as environmental risk factors for rheumatoid arthritis (RA). EBV infections are known to be nearly ubiquitous in adults, life long, and asymptomatic. EBV infection status has been determined using circulating levels of IgG antibodies to Epstein Barr viral capsid antigen (VCA), nuclear antigen 1 (EBNA-1), and early antigen (EA). Prior studies have shown that patients with established RA have altered immune responses to EBV; however, the presence and role(s) for this altered immune response has not been fully understood in the autoantibody positive early period before clinically apparent RA develops. We hypothesize that EBV infection, as evidenced by an altered anti-EBV antibody response in preclinical RA, can play an important role in driving the expansion of RA-related autoimmunity.

Methods

Subjects

- Study cohort included 83 subjects with RA (termed cases) using 1987 ACR guidelines and stored serum samples available through the Department of Defense Serum Repository (DoDSR) that include the pre and post RA diagnosis period. Controls (n=83) were matched to RA cases based on age, race, sex, region, and timing of blood draw.
- Subjects' sera were tested for 5 anti-EBV antibodies (EBNA-1-IgG, VCA-IgG, EA-IgG, VCA-IgA, and EA-IgA), 7 RA-related autoantibodies (RF-neph, -IgA, -IgM, -IgG, CCP2, CCP.3, CCP3.1), 22 cytokine/chemokine markers, and 36 anti-citrullinated peptide antibody reactivities (ACPAs).

Statistical Methods

- EBV levels were determined using ELISA based assays and were compared by RA case and control status using $\chi 2$ or t-test.
- Random forest was used to determine which variables are important in distinguishing RA cases from controls. Untransformed biomarker levels for modeling were pre-selected based on bivariate association with RA case versus control outcome using Mann-Whitney test ($p< 0.20$).
- Individuals' trajectories over time were log-transformed.
- Linear mixed models were used to determine if there was a difference in the levels of EBV/RA markers over-time between RA cases and controls. An interaction term was included to test whether there is a change in biomarkers over-time that differs by group.
- Joint modeling was done to test for correlations between EBV markers and RA markers over-time between cases and controls treating EA IgG or IgA as the outcome and RA markers as the predictor variables.

Results

Table 1:Cohort Demographics

	RA Case	Control	p value
N	83	83	
Samples pre-diagnosis: N	242	242	1.00
Samples post-diagnosis: N	48	48	1.00
Samples per subject pre-diagnosis: mean (range)	2.9 (1-4)	2.9 (1-4)	1.00
Samples per subject post-diagnosis: mean (range)	1 (1-1)	1 (1-1)	1.00
Age at diagnosis: mean ± SD	39.9 ± 10.0	40.0 ± 9.9	0.98
Sex: % male	49 (59.0%)	49 (59.0%)	1.00
Race: % white	57 (68.7%)	57 (68.7%)	1.00
RF IgA: ever: % positive*	**58 (69.9%)**	**10 (12.1%)**	**0.0001**
RF IgG: ever % positive*	**38 (45.8%)**	**12 (14.5%)**	**<0.0001**
RF IgM ever: % positive*	**56 (67.5%)**	**10 (12.1%)**	**<0.0001**
RF neph. ever: % positive*	**56 (67.5%)**	**12 (14.5%)**	**<0.0001**
CCP2: ever % positive*	**56 (67.5%)**	**0 (0.0)**	**<0.0001**
CCP3: ever % positive*	**56 (67.5%)**	**3 (3.6)**	**<0.0001**
CCP3.1: ever % positive*	**60 (72.3%)**	**14 (16.9)**	**<0.0001**

% ever positive: subjects had at least one positive sera sample
$p<0.05$ considered significant by X^2 or t-test

Table 2: Anti-EBV Antibodies Levels

	RA Case	Control	p value
EBNA-1 IgG (OD) positive ever: N (%yes)	82 (98.8%)	82 (98.8%)	1.00
EBNA-1 IgG (OD) level: *mean ± SD	**0.84 ± 0.27**	**0.77 ± 0.21**	**0.05**
pre-RA visits: *mean ± SD	0.86 ± 0.29	0.79 ± 0.23	0.06
post-RA visits: †mean ± SD	0.73 ± 0.25	0.68 ± 0.20	0.27
VCA IgG (ISR) positive ever: N (%yes)	79 (95.2%)	81 (97.6%)	0.68
VCA IgG (ISR) level: *mean ± SD	**4.44 ± 1.68**	**3.88 ± 1.27**	**0.02**
pre-RA visits: *mean ± SD	**4.39 ± 1.68**	**3.82 ± 1.24**	**0.01**
post-RA visits: †mean ± SD	4.86 ± 1.91	4.32 ± 1.71	0.15
EA IgG (ISR) positive ever: N (% yes)	**28 (33.7%)**	**9 (10.8%)**	**0.0004**
EA IgG (ISR) level: *mean ± SD	**0.82 ± 0.72**	**0.49 ± 0.28**	**0.0002**
pre-RA visits: *mean ± SD	**0.82 ± 0.72**	**0.49 ± 0.29**	**0.0002**
post-RA visits: †mean ± SD	**0.86 ± 0.61**	**0.56 ± 0.31**	**0.01**
VCA IgA (ISR) positive ever: N (% yes)	6 (7.2%)	2 (2.4%)	0.28
VCA IgA (ISR) level: *mean ± SD	0.34 ± 0.22	0.29 ± 0.19	0.14
pre-RA visits: *mean ± SD	0.34 ± 0.23	0.29 ± 0.19	0.17
post-RA visits: †mean ± SD	0.32 ± 0.24	0.25 ± 0.17	0.09
EA IgA (ISR) positive ever: N (% yes)	51 (61.5%)	43 (51.8%)	0.21
EA IgA (ISR) level: *mean ± SD	**0.97 ± 0.49**	**0.79 ± 0.46**	**0.02**
pre-RA visits: *mean ± SD	**1.02 ± 0.50**	**0.82 ± 0.48**	**0.01**
post-RA visits: †mean ± SD	0.78 ± 0.77	0.68 ± 0.63	0.48

*mean level calculated for each subject using all visits then compared across case/control status
†only 1 sample max per subject post-RA. 94 subjects have post-RA sample.
$p<0.05$ considered significant by X^2 or t-test

Figure 1: Random Forest Analysis Identifies Anti-EBV Antibodies

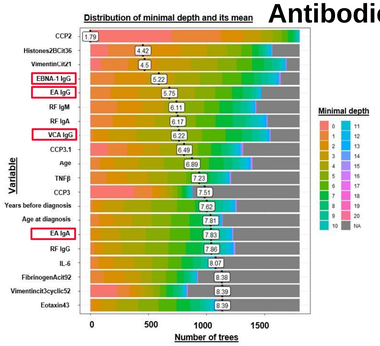

Figure 1: A total of 2,000 decision trees were run and the top 20 important variables for classifying RA cases versus controls involved are presented. Minimal depth describes where variable appears in decision trees where lower the number the closer to that variable is to root node. Red indicates root node. Average depth of each variable is indicated.

Figure 2: Linear Mixed Modelling of Anti-EBV Antibodies

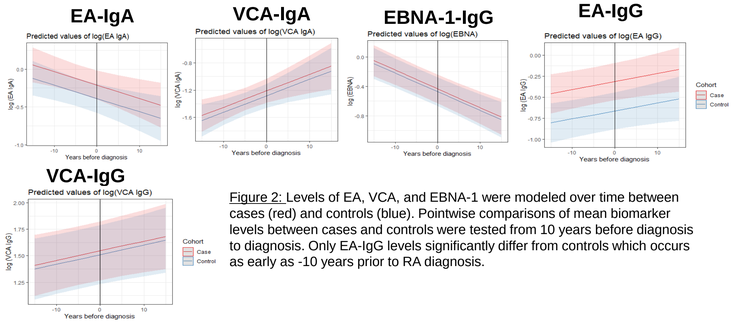

Figure 2: Levels of EA, VCA, and EBNA-1 were modeled over time between cases (red) and controls (blue). Pointwise comparisons of mean biomarker levels between cases and controls were tested from 10 years before diagnosis to diagnosis. Only EA-IgG levels significantly differ from controls which occurs as early as -10 years prior to RA diagnosis.

Figure 3: Linear Mixed Modelling of CCP and RF antibodies

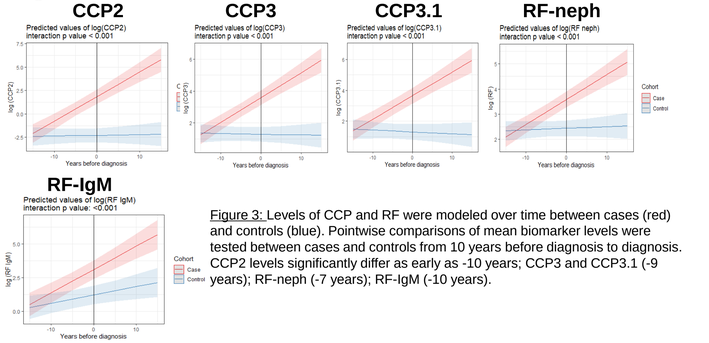

Figure 3: Levels of CCP and RF were modeled over time between cases (red) and controls (blue). Pointwise comparisons of mean biomarker levels were tested between cases and controls from 10 years before diagnosis to diagnosis. CCP2 levels significantly differ as early as -10 years; CCP3 and CCP3.1 (-9 years); RF-neph (-7 years); RF-IgM (-10 years).

Figure 4: Joint modeling of EA (IgG or IgA) with other RA markers

Correlation of subject-specific RA marker rate of change with subject-specific log EA IgA rate of change (standard error)	Random slope for each outcome [cases]	P value	Random slope for each outcome [controls]	P value
Log CCP2	0.089 (0.269)	0.741	-0.202 (0.333)	0.547
Log CCP3	-0.084 (0.268)	0.754	-0.182 (0.347)	0.601
Log CCP3.1	-0.032 (0.267)	0.905	0.334 (0.232)	0.154
Log RF neph	0.039 (0.356)	0.913	-0.294 (0.181)	0.108
Log RF IgM	0.112 (0.310)	0.708	-0.024 (0.210)	0.908

Correlation of subject-specific RA marker rate of change with subject-specific log EA IgG rate of change (standard error)	Random slope for each outcome [cases]	P value	Random slope for each outcome [controls]	P value
Log CCP2	0.461 (0.184)	0.014	0.893 (0.073)	<0.001
Log CCP3	0.436 (0.182)	0.019	0.588 (0.250)	0.021
Log CCP3.1	0.334 (0.195)	0.090	0.543 (0.173)	0.002
Log RF neph	0.557 (0.191)	0.005	0.031 (0.175)	0.860
Log RF IgM	0.512 (0.183)	0.007	-0.256 (0.178)	0.150

Figure 4: Joint modeling was done to determine the relationship between RA markers and anti-EA antibody levels in RA cases and controls. Correlation significance was determined by t-tests ($p<0.05$) Positive slope indicates positive correlation between the markers negative slope values indicate negative correlation. The relationship between RF neph, RF-IgM and EA-IgG is significant in RA cases but not in controls suggesting an endotype of RA.

Conclusions

Our study suggests that several anti-EBV antibody levels are significantly elevated in the preclinical time of RA. In addition, altered EBV antibody titers suggests that this virus may contribute to a specific endotype of preclinical RA, particularly with RF neph/RF-IgM and EA-IgG.

Disclosures

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