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P B Ferrell, C T Aitcheson, G R Pearson, E M Tan

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Research Article

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Seroepidemiological Study of Relationships between Epstein-Barr Virus and Rheumatoid Arthritis

P. BRENT FERRELL, CAROL T. AITCHESON, GARY R. PEARSON, and ENG M. TAN, *Division of Rheumatic Diseases, University of Colorado Health Sciences Center, Denver, Colorado 80262; Section of Microbiology, Mayo Foundation, Rochester, Minnesota 55901*

A B S T R A C T To elucidate the relationship between Epstein-Barr virus (EBV) and rheumatoid arthritis (RA), we measured antibodies to RA-associated nuclear antigen (anti-RANA) and three other EBV-related antigens in the sera of RA patients and controls. Our study groups consisted of 89 patients with definite or classical RA, mean age 56, male/female ratio 47:42; and 53 normal and osteoarthritis controls, mean age 51, male/female ratio 25:28. In addition to anti-RANA, we measured antibodies to viral capsid antigen (anti-VCA), early antigen (anti-EA) and EBV-associated nuclear antigen (anti-EBNA).

Anti-RANA was detected in 71% of RA patients but in only 6% of controls. Elevated anti-VCA titers ($>1:160$) were more common in RA patients than controls, 31% compared with 15%. The geometric mean titer of anti-VCA was significantly higher in the RA group, 133 compared with 58. Anti-EA was present in 53% of RA patients but only 19% of controls. Anti-EA in elevated titers ($>1:20$) was present in 26% of RA patients but only 7% of controls. Characterization of the anti-EA antibodies revealed that the RA patients reacted primarily with the diffuse component, whereas the majority of the controls reacted with the restricted component of the EA complex. In contrast, the frequencies, distributions, and geometric mean titers of anti-EBNA were not significantly different between the two groups. Correlative analysis of these antibodies showed highly significant relationships between anti-VCA and anti-EA, and anti-RANA and anti-EBNA in the RA group. These data are compatible with the interpretation that RA patients have either more active EBV infections than controls or an altered regulation of their immune response to this infectious agent.

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INTRODUCTION

Recently, the demonstration that patients with seropositive rheumatoid arthritis (RA)¹ have a high frequency of antibodies to a nuclear antigen present only in Epstein-Barr virus (EBV)-transformed lymphocytes has renewed interest in the relationship between EBV and RA. These antibodies, initially reported as RA precipitin, were detected in immunodiffusion using an extract from the WiL-2 lymphoblastoid cell line. They were shown to be present in 67% of seropositive RA patients but present in much lower frequencies in other connective tissue diseases. Subsequently, indirect immunofluorescent studies showed that RA precipitin-containing prototype sera gave a characteristic speckled nuclear staining pattern on the cultured lymphocytes used to prepare the extract. Certain other cultured cell lines, human organ sections, and normal human lymphocytes did not display this pattern of nuclear staining (1). Further investigations demonstrated that the antigen recognized by RA-precipitin-containing sera, later called RA-associated nuclear antigen (RANA), was restricted to EBV-transformed cells. EBV-transformed cell lines shown to contain RANA included the Raji, WiL-2, and HCL-3/B95-8 lines (all of human origin), the 531-H line (of owl monkey origin), and the B95-8 and 1605-S lines (both of cotton-top marmoset origin) (2). Evidence from these studies also suggested RANA was distinct from the previously described EBV-associated nuclear antigen

¹ Abbreviations used in this paper: BSS, balanced salt solution; D component, diffuse component of the early antigen complex; EA, early antigen; EBNA, Epstein-Barr virus associated nuclear antigen; EBV, Epstein-Barr virus; GMT, geometric mean titer; R component, restricted component of the early antigen complex; RA, rheumatoid arthritis; RANA, RA-associated nuclear antigen; RF, rheumatoid factor; VCA, viral capsid antigen.

(EBNA). More recently, other investigators have provided still further data linking EBV to RA. Slaughter et al. (3) showed that lymphocytes taken from RA patients spontaneously transformed into continuous cell lines more often and more quickly than lymphocytes from normals. In addition, infection with EBV induced these lymphocytes to produce significantly more IgM rheumatoid factor. Bardwick et al. (4) extended these findings by showing that T lymphocytes from RA patients demonstrated diminished control over EBV-induced proliferation of autologous B lymphocytes. This was manifested as a more rapid EBV-transformation of RA compared with control peripheral blood mononuclear cells.

A close relationship between EBV and three diseases, infectious mononucleosis, Burkitt's lymphoma, and nasopharyngeal carcinoma, is now generally accepted. Such relationships were initially suggested by seroepidemiological studies of the three diseases and later confirmed by studies demonstrating EBV genomes in the involved tissues (5). Based on these previous experiences, it seemed important to carry out similar investigations in RA patients. We report here a seroepidemiological study of EBV-related antibody responses in RA patients and controls of similar age and sex ratio. The antibody titers obtained are compared both within and between groups to uncover any possible relationships. It is shown that RA patients have elevated titers of anti-VCA and a higher frequency of antibodies to early antigen (anti-EA), especially those directed to the diffuse component of the EA complex (D component). More importantly it is shown that titers of anti-VCA and anti-EA are significantly correlated only in RA patients. These results are discussed with reference to what is known about such antibody responses in EBV-related diseases.

METHODS

Sera. Sample sera were collected from 89 patients with definite or classical RA and 53 controls. Patients in the RA group were classified according to American Rheumatism Association criteria (6). This group had a mean age of 56 with a male/female ratio of 47:42. The control group consisted of 37 normal donors, mean age of 40 yr. and male/female ratio 17:20, and 16 patients with degenerative joint disease, mean age of 67 and male/female ratio 8:8. The mean age of the entire control group was 51 yr, with a male/female ratio of 25:28. Drug therapy at the time of serum collection was recorded for 85 of 89 patients in the RA group. 28 of 85 (33%) patients were taking prednisone, and 4 of 85 cyclophosphamide. Only 4 of the 28 prednisone-treated patients were taking daily doses greater than 7.5 mg. Therapy in the control group consisted predominantly of nonsteroidal anti-inflammatory drugs for degenerative joint disease patients. After collection, sera were heat-inactivated at 56°C for 30 min and stored at -20°C.

Cell lines and cell extracts containing RANA. WiL-2 (7) and Ramos (8) are continuously growing human lymphoid cell lines. WiL-2 cells carry the EBV genome and express EBNA, whereas Ramos cells do not carry the EBV genome and thus do not express EBNA. Both cell lines were grown in Auto-

Pow medium (Flow Laboratories, Inc., Rockville, Md.) supplemented with vitamins, nonessential amino acids, sodium pyruvate, glutamine, and antibiotics (50 U/ml penicillin and 50 µg/ml streptomycin). Heat-inactivated fetal bovine serum was added to a final concentration of 10%.

A RANA-containing cell-free extract was prepared as described by Alspaugh et al. (3). Briefly, WiL-2 cells were grown to a density of ~10⁶ cells/ml and harvested by centrifugation at 200 g for 5 min at 4°C. Cells were washed once in medium without fetal bovine serum. The final cell pellet was resuspended with an equal volume of sonicating buffer containing 25 mM sucrose, 4 mM CaCl₂ and 10 mM Na₂HPO₄/NaH₂PO₄, pH 6.2. This suspension was sonicated for four 15-s cycles at maximum output with a model W-220F sonicator (Heat Systems-Ultrasonics, Plainview, N. Y.). The sonicate was centrifuged at 40,000 rpm in a type 40 rotor (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.) for 30 min at 4°C. After centrifugation, the supernate (RANA-containing extract) was recovered and frozen in aliquots at -20°C. The same batch of extract was used to evaluate all the sera in this study (anti-RANA assay below).

Rheumatoid factor (RF) determination. RF was quantitated by the 12-tube latex agglutination method (Calbiochem-Behring Corp., American Hoechst Corp., San Diego, Calif.). Sera in each group were tested in doubling dilutions from 1:10 to 1:40,960.

Antibody assays. Anti-RANA antibodies were detected by a modification of the Ouchterlony double diffusion method (9). Plastic petri dishes (1 × 5 cm) containing 5 ml of 0.4% agarose (wt/vol) (Seakem, FMC Corp., Rockland, Me.) and 0.1% sodium azide (wt/vol) were used. Fourfold dilutions of sera were prepared starting with undiluted serum and diluting to 1:64. 100 µl of each dilution were placed in wells surrounding a well containing 100 µl of WiL-2 extract. Wells were 4 mm in Diam and 3 mm apart in nearest circumference. A reference serum known to contain only anti-RANA was used in wells adjacent to the test sera to establish identity or nonidentity of precipitin lines. Precipitin lines usually appeared in 24–48 h but were examined for up to 96 h at room temperature. Though no specific experiments were done in this study to determine the influence of RF on serum titers of anti-RANA, previous reports have found no significant differences in anti-RANA titers before and after absorption of RF (1, 10). Similarly, anti-EBNA and anti-VCA assays (below) were reportedly not affected by absorption of RF (10).

Anti-EBNA antibodies were demonstrated by the anti-complement immunofluorescent technique (11). WiL-2 or Ramos cells in exponential growth were harvested by centrifugation at 200 g for 5 min at 4°C. They were washed once with cold balanced salt solution (BSS) containing 136 mM NaCl, 5 mM KCl, 1 mM CaCl₂, 0.8 mM MgSO₄, 10 mM Na₂HPO₄/KH₂PO₄, pH 6.9, then resuspended in cold BSS at a concentration of 15 × 10⁶ cells/ml. 10 µl of this WiL-2 cell suspension were placed on each of nine spots of a coated multi-spot slide (Cel-Line Associates, Minotola, N. J.). After 1 min, the cells tended to settle and the excess buffer could be aspirated with a Pasteur pipette. The cells and buffer remaining on the spot were left to air-dry. A Ramos cell smear was prepared identically on another spot of each slide and served as the EBV-negative cell control. The slides were fixed for 5 min in a 1:1 mixture of methanol:acetone at -20°C. Cell smears were always prepared fresh for each batch of sera tested. Two-fold dilutions of sera were prepared beginning with a 1:4 dilution. A 1:4 dilution of test serum was always screened on the Ramos cell smear for the presence of non-EBNA complement-fixing antinuclear antibodies. After preparation of the serum dilutions, cell smears were stained by the following steps. First, serum dilutions were incubated with the cell smears for 30 min in a moisture chamber and then removed

by a 5-min wash in BSS with gentle agitation. Second, a serum previously determined to be devoid of anti-EBNA antibodies and diluted 1:5 was added to the cell smears to act as a complement source. After 30 min incubation in a moisture chamber, the slides were washed with BSS for 5 min as before and fluorescein-conjugated goat anti-human C3 anti-serum (Hyland Laboratories, Costa Mesa, Calif.) diluted 1:30 was added. After 45 min incubation in a moisture chamber, the slides were washed in BSS for 30 min again with gentle agitation. Finally, a drop of BSS-glycerol 1:1 was placed on the slide and a coverslip applied.

The stained slides were examined at $\times 400$ with a Leitz Ortholux II incident light microscope with a 200 watt mercury light source, KPF90 exciter filter and K530 barrier filter (E. Leitz, Inc., Rockleigh, N. J.). The antibody titer was defined as the reciprocal of the last dilution showing definite speckled nuclear fluorescence in a majority of cells. The anti-EBNA titer for each slide was determined independently by two observers (Dr. Ferrell and Dr. Aitcheson). Observers agreed on titers greater than 80% of the time. If no agreement could be reached, the assay was repeated and the serum titer taken as the median titer of the four separate observations (two for each observer). Any serum that was positive at a 1:4 dilution on Ramos cells was then titrated on Ramos and WiL-2 cell smears in parallel to determine the titer on each cell line. If the titer on WiL-2 cells was less than that on Ramos cells, the serum was considered to have interfering antinuclear antibody making it difficult to interpret the findings on the EBV-positive WiL-2 cells.

The anti-VCA and anti-EA studies were performed in Dr. Pearson's laboratories using coded sera. Anti-VCA titers were detected on acetone-fixed smears of P3HR-1 cells (12). These smears contained 10–15% VCA-positive cells. Anti-VCA titers were determined with a goat anti-human IgG (H and L chain) fluorescent reagent (Hyland Laboratories).

Anti-EA antibodies were detected on acetone-fixed smears of EBV-superinfected Raji cells as described (13). These smears contained 20–25% EA-positive cells and less than 1% VCA-positive cells as determined with standard sera. To determine whether antibodies were directed to the D component or restricted component of EA (R component), sera were also titrated on cells fixed in methanol, which destroys the R component (13). Sera for both anti-VCA and anti-EA were titrated in fourfold dilutions beginning with a 1:10 dilution. The slides were examined with an American Optical fluorescence microscope by Dr. Pearson without previous knowledge of the anti-RANA or anti-EBNA titers.

Geometric mean titers of both groups of sera for each antibody assay were determined by assigning an arbitrary titer of one to any sera having a titer less than the lowest dilution tested.

Statistics. Tests for determination of statistical significance included Student's *t* test and the chi-square test for contingency tables (14).

RESULTS

RF. RF was detected in 85 of 89 (96%) of RA patients. Titers ranged from 1:20 to 1:40,960.

Anti-RANA. As shown in Fig. 1, there was a clear difference in the frequency of anti-RANA titers between the control group and the rheumatoid arthritis group. Only 6% of the control group was found to have antibody to RANA vs. 71% of the RA group. Titers of anti-RANA in RA patients were distributed throughout the range measured with 19% of RA patients having titers greater than or equal to 1:64. Only one person in

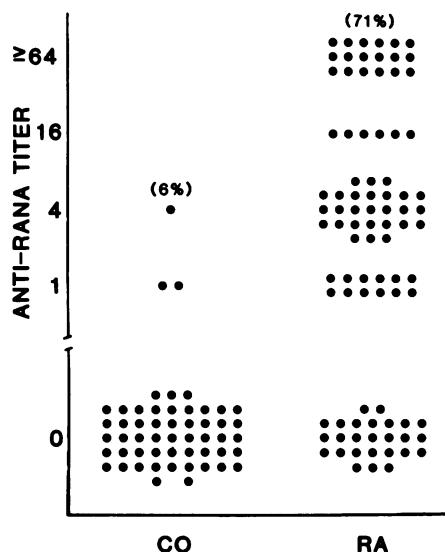


FIGURE 1 Distribution of serum titers of anti-RANA in RA patients and controls (CO). Each point represents the reciprocal of the highest dilution (titer) of each serum which produced a precipitin line against WiL-2 extract in a double immunodiffusion assay. The percentage of sera with anti-RANA is shown for each group at the top of the figure. 71% of RA sera had anti-RANA compared with only 6% of CO sera.

the control group had anti-RANA antibodies detectable in diluted serum.

Anti-EBNA. It can be seen from Table I that antibodies to EBNA were present both in the control and the RA groups at similar frequencies, 89 and 97%, respectively. These frequencies closely approximate those of most normal populations (15). Presence or absence of anti-EBNA in nine sera of the RA group and two sera of the control group could not be definitely established due to interfering antinuclear antibody as defined in Methods. These sera were therefore excluded from subsequent calculations of anti-EBNA titers. Trial inclusion of the sera in question, as all positive or all negative, did not significantly change the final frequencies in either group. The distributions of anti-EBNA titers in both groups were compared in Fig. 2. No difference in the two patterns of distributed titers were seen. Percentages of sera with elevated titers ($>1:256$) were 18% in RA patients and 19% in controls; and GMT were 119 in RA patients and 112 in controls. Though not shown, GMT for the two control subgroups, normal donors and degenerative joint disease patients were not significantly different from the GMT of anti-EBNA of the entire control group.

Anti-VCA. As shown in Table I, frequencies of anti-VCA in both groups were similar to the frequencies of anti-EBNA. However, as shown in Fig. 3, the distributions of titers were quite different. The percentage of sera with elevated titers ($>1:160$) in the RA group was significantly higher than the control group, 31% compared with 15%, $P < 0.01$. The GMT were also signifi-

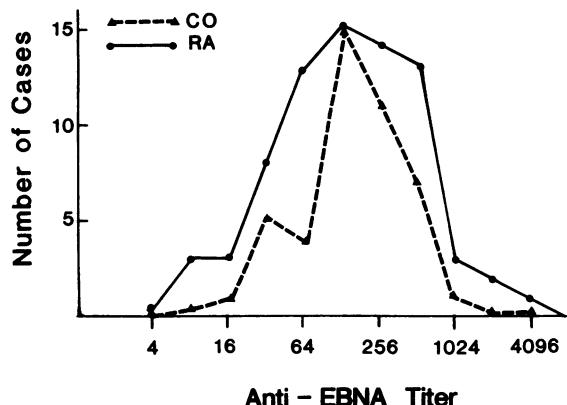


FIGURE 2 Distribution of serum titers of anti-EBNA in RA patients and controls (CO). Each point represents the number of sera with the indicated titer (titer is defined as the reciprocal of the highest serum dilution showing detectable fluorescence in the anti-complement immunofluorescence assay). Serum titers of anti-EBNA in both groups are distributed similarly.

cantly different, 133 compared with 58, $P < 0.005$. As for anti-EBNA, the GMT of anti-VCA for the control subgroups were not significantly different from the GMT of the entire control group. Only three sera in the RA group and no sera in the control group contained antibodies that interfered with interpretation of the anti-VCA assay.

Anti-EA. Frequencies of anti-EA antibodies were strikingly different between the two groups (Table I). The RA group had a significantly greater percentage of positive sera than the control group, 53% compared to 19%, $P < 0.001$. This difference was maintained when the percentages of sera with elevated titers ($> 1:20$) were compared, 26% compared with 7%, $P < 0.01$. Geometric mean titers in both groups were quite low due to the excessive numbers of titers less than 1:10. (Due to the non-Gaussian distribution of the titers in these two groups, the differences could not be tested for significance by the Student's t test). Analy-

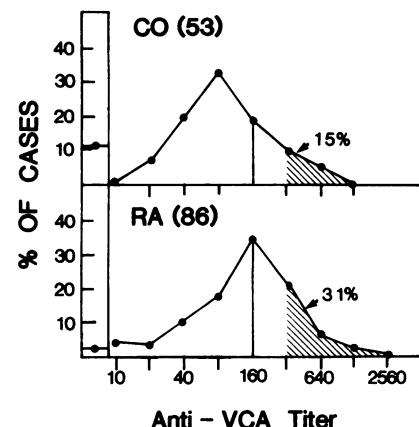


FIGURE 3 Distribution of serum titers of anti-VCA in RA patients and controls (CO). Points represent percent sera with the indicated titers (titer is defined as in Fig. 2 by an indirect immunofluorescence assay). Percentage of sera with titers less than 1:10 is indicated by the points in the enclosed area at the left of the graph. Cross-hatched areas represent percent sera in each group with significantly elevated titers ($> 1:160$). 31% of RA sera had elevated titers compared with 15% of CO sera. These frequencies were significantly different ($P < 0.01$).

sis of anti-EA specificities also yielded very different results between the two groups (Table II). 36 of 46 (80%) RA sera contained antibodies to the D component of the early antigen complex, whereas only 2 of 10 (20%) control sera were positive for antibodies to this component. These values were significantly different ($P < 0.001$).

Correlation of antibody titers and RF titers. Correlation of antibody titers were shown in Figs. 4–6. Fig. 4 shows that in RA patients the presence of anti-EA occurred only with elevated titers of anti-VCA and that these titers were significantly correlated ($r = 0.64$, $P < 0.001$). The same antibodies in the control group showed no significant correlation ($r = 0.24$, $P > 0.1$). Fig. 5 shows no significant relationship between anti-VCA and anti-EBNA in either group. Fig. 6 shows a

TABLE I
Antibody Responses to EBV-related Antigens in RA Patients and Controls

Antibody	Group	Number studied	Percentage with antibody	Percentage with elevated titers*		Geometric mean titer
				%	%	
Anti-EBNA	RA	80	97	18	19	119
	Control	51	89			
Anti-VCA	RA	86	97	31	15	133
	Control	53	89			
Anti-EA	RA	86	53	26	7	6
	Control	53	19			

* Titters considered elevated: anti-EBNA $> 1:256$, anti-VCA $> 1:160$, anti-EA $> 1:20$.

† Not comparable by the Student's t test (see text).

TABLE II
Antibody Responses to the D Component and R Component of the EA Complex

Group	Total number with anti-EA	Percentage with antibodies to the D component	Percentage with antibodies to the R component
		%	%
RA	46	80	20
Control	10	20	80

$P < 0.001$

strong correlation between anti-RANA and anti-EBNA ($r = 0.58, P < 0.001$) but no significant correlation of anti-RANA with either anti-VCA or anti-EA.

As seen in Table III rheumatoid factor showed no significant correlation with any of the antibody titers in the RA group though there was a trend to higher RF titers in the sera containing anti-RANA.

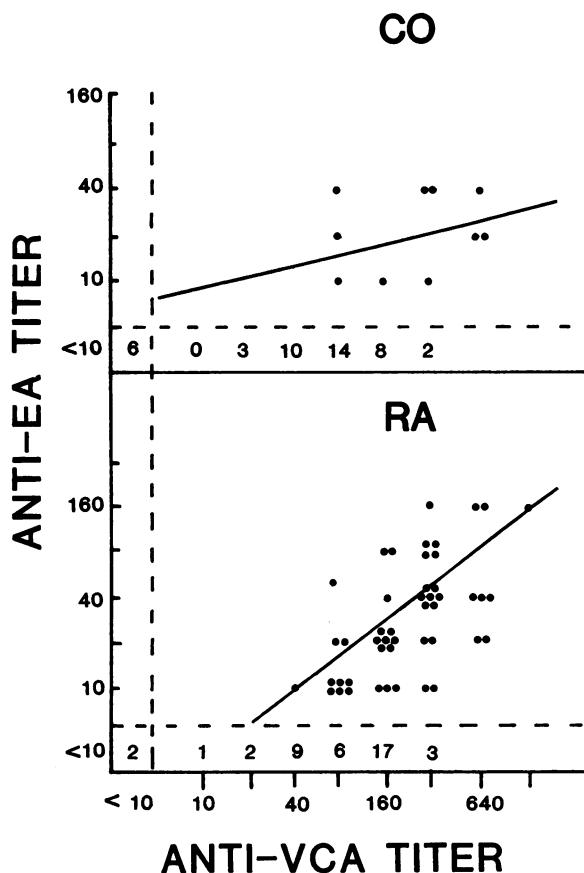


FIGURE 4 Correlation of serum titers of anti-VCA and anti-EA by the method of least squares. Only sera having an anti-EA titer equal to or greater than 1:10 were included in the determination of the correlation coefficients. Sera with anti-EA titers less than 1:10 at each titer of anti-VCA are represented by the numbers below the dashed lines. The anti-EA and anti-VCA titers are significantly correlated in the RA group ($r = 0.64, P < 0.001$) but not in the control (CO) group ($r = 0.24, P > 0.1$).

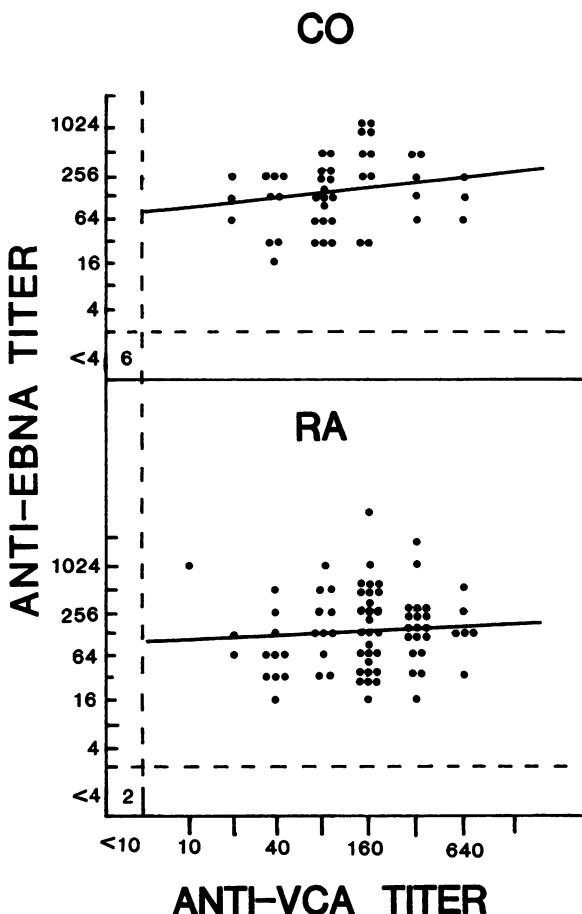


FIGURE 5 Comparison of anti-VCA and anti-EBNA titers in RA and control (CO) sera. Only sera with anti-VCA titers equal to or greater than 1:10 were included in determination of the correlation coefficients. Numbers below the dashed lines denote sera with anti-VCA titers less than 1:10. There was no significant correlation noted in either group ($r = 0.26, P > 0.1$ in CO and $r = 0.06, P > 0.1$ in RA group).

DISCUSSION

This study of antibody responses to EBV in rheumatoid arthritis has demonstrated several interesting features. The frequencies of anti-VCA in RA and controls were similar, indicating that as far as infection or exposure to the virus was concerned, RA patients did not appear to have different exposure experiences. However, a higher percentage of RA patients had elevated titers of anti-VCA compared with controls. The most striking feature was the increase in both overall frequency, and frequency of elevated titers of anti-EA in RA compared to controls. Not only these elevated anti-VCA and anti-EA titers but also anti-RANA and anti-EBNA titers appeared to be independent of RF as shown by the lack of correlation between antibody titers and RF titers.

In the early seroepidemiological studies of antibodies to EBV antigens, it was rapidly noted that EBV was a ubiquitous virus and that antibodies to VCA were

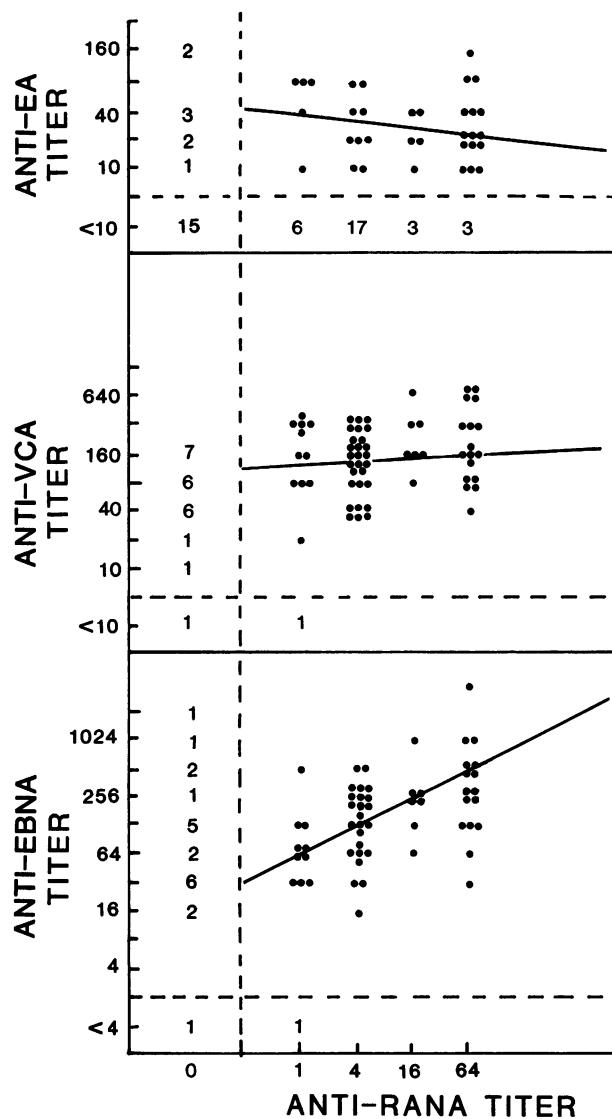


FIGURE 6 Comparison of anti-RANA titers with titers of anti-EBNA, anti-VCA, and anti-EA in RA patients. Only sera that had antibodies to RANA and had an anti-EBNA titer equal to or greater than 1:4 were included in the determination of the correlation coefficients. Other sera are indicated by the numbers below or behind the dashed lines. Anti-EBNA titers were significantly correlated with anti-RANA titers ($r = 0.58$, $P < 0.001$) but not with anti-EA ($r = -0.16$) or anti-VCA ($r = 0.09$) titers.

present in a very high percentage of both normal populations and patients with diseases thought to be related to EBV. These studies showed that simply detecting antibodies to VCA in various diseases was clearly not sufficient to prove an etiologic role for EBV. Subsequently, investigators demonstrated that it was essential to determine the titers of these antibodies in various diseases and to compare them with those observed in appropriate control groups. As in the study reported here, elevated titers of anti-VCA have been shown to

TABLE III
Correlation Coefficients between EBV-related Antibody Titers and RF Titers in RA Patients

	Anti-RANA	Titers of anti-EBNA	Anti-VCA	Anti-EA
RF titers	$r = 0.23^*$	$r = 0.20^*$	$r = -0.06^*$	$r = -0.19^*$

* Not significant.

be present in significantly higher frequencies in the EBV-related diseases, infectious mononucleosis, Burkitt's lymphoma, and nasopharyngeal carcinoma, than in controls (5). Anti-VCA titers have also been shown to be elevated, albeit inconsistently, in several other diseases including sarcoidosis, lymphomas, and systemic lupus erythematosus.

Despite the higher anti-VCA titers in the RA patients observed here as well as in the diseases just noted, investigators such as Henle (5) have emphasized the need to obtain additional serologic evidence before attempting to link EBV with a given disease. This additional evidence in the case of infectious mononucleosis, Burkitt's lymphoma, and nasopharyngeal carcinoma came from studies on antibodies to the EA complex showing distinct differences between these diseases and controls. The data in the present study show both an increased frequency of anti-EA and higher titers of this antibody in RA compared with controls. It is also interesting that the predominant antibody is directed to the D component. Antibodies to the D component are detected in nasopharyngeal carcinoma and transiently in infectious mononucleosis. In Burkitt's lymphoma, antibody to the R component is present but not antibody to the D component (5). As in this study, in normal patients who may rarely have anti-EA, the predominant antibody is usually directed to the R component (16).

Our studies show that the frequency and geometric mean titer of anti-EBNA in RA patients are not significantly different from controls. In this respect, RA patients are different from patients with ataxia-telangiectasia, who also have heightened immune responses to VCA and EA but depressed responses to EBNA (17, 18). It has been suggested that the low titers of anti-EBNA in ataxia-telangiectasia as well as in certain other diseases with heightened reactivities to VCA may be related to a generalized cellular immune dysfunction (19). Our correlative analysis of anti-EBNA and anti-RANA revealed a significant relationship ($r = 0.58$, $P < 0.001$). Catalano and associates (15) have also found a close relationship between the two antibodies. They demonstrated that anti-EBNA antibody titers were significantly higher in sera that were positive for anti-RANA than in sera negative for the latter antibody. Further studies by these investigators (20) have shown that anti-RANA appears in patients with infectious mono-

nucleosis several months after the initial infection and titers of anti-RANA rise concomitantly with anti-EBNA. As in other studies described above, these data give further evidence suggesting a positive relationship between EBV and RA. Recently, Ng and associates (21) have confirmed the high frequency (93%) of anti-RANA in RA compared with normal controls (16%) and have further observed that in seronegative (rheumatoid factor negative) RA there is also a high frequency of anti-RANA (95%).

This seroepidemiological study of RA has shown that RA patients do not appear to have either an increased or decreased frequency of exposure to EBV infections. From the pattern of antibody responses, there is also no suggestion that RA patients in general have delayed primary EBV infections that might account for their high frequency of elevated anti-EA titers. With the exception of two patients devoid of anti-EBV antibodies, all RA patients had anti-EBNA antibody, known to be a late sequela of prior EBV infection. Further, it is unlikely that these results are attributable to a general suppression of the immune system since anti-EBNA titers in the RA group are normal (unlike ataxia-telangiectasia), and the predominant immunosuppressive drug prednisone was usually employed in low doses. One interpretation of the present observations is that RA patients have more active infections than normals, as demonstrated by the higher titers of anti-VCA and the higher frequency and titers of anti-EA. These active infections in RA patients may be related to a defect in T cell regulation of B cells latently infected with EBV. Preliminary evidence for such a defect has come from the studies of Bardwick et al. (4), who have shown that T cells from RA patients demonstrated diminished control over EBV-induced proliferation of autologous non-T lymphocytes. Several laboratories have now demonstrated some connection between EBV and RA. The lead afforded by immunological and seroepidemiological observations should provide a basis for determining possible etiologic and pathogenetic mechanisms.

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