

A seroepidemiological study of cytomegalovirus and Epstein-Barr virus in rheumatoid arthritis and sicca syndrome

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SUMMARY Antibodies to cytomegalovirus (CMV) and Epstein-Barr virus capsid antigen (EBVCA) were examined in 41 patients with rheumatoid arthritis (RA), 26 patients with primary sicca syndrome, and 26 healthy subjects of similar age and sex. IgG antibody titres to EBVCA and CMV were similar in all three groups, apart from a trivial increase of antibodies to EBVCA in RA. False positive IgM anti-CMV antibodies detected in serum from one patient with sicca syndrome and 20 patients with RA were shown to be due to rheumatoid factors. These data did not support recent suggestions that patients with these diseases showed exaggerated immunological responses to either virus and emphasised the need to incorporate adequate laboratory and disease controls when seroepidemiological studies are performed on sera containing rheumatoid factors and autoantibodies.

The Epstein-Barr virus (EBV) and cytomegalovirus (CMV) are candidate agents for the aetiology of rheumatoid arthritis (RA) and sicca syndrome (SS). Both viruses infect salivary glands and both, once infection has been established, persist for life and could act as triggers for the immunological abnormalities seen in these diseases.

Seroepidemiological evidence for the involvement of EBV in RA has been suggested by the finding of higher antibody titres to Epstein-Barr viral capsid antigen (EBVCA),¹⁻³ Epstein-Barr nuclear antigen (EBNA),⁴ and early antigen.^{2,3} However, in these studies the titre differences were small (usually about one doubling dilution) and there was disagreement about which of the EBV determined antigens represent the targets for this apparently exaggerated response. There has been agreement that antibodies to rheumatoid arthritis nuclear antigen (RANA), initially described as a precipitin reaction characteristic of RA,⁵ are significantly raised, with values ranging from about $6 \times \log_2$ in our own studies,⁶ to $16 \times \log_2$ ³ compared with controls. Recent findings that RANA determinants appear to

reside on the EBNA polypeptide^{7,8} suggest that the higher levels of anti-RANA in RA sera could represent a relatively specific immune response to part of an EBV related polypeptide rather than reflecting a generalised hyper-responsiveness to EBV. In the present study we examine this possibility by comparing titres of antibodies to EBVCA in our patients with titres in normal subjects to see if, like anti-RANA, anti-VCA titres are raised in our patients.

A possible role for CMV in sicca syndrome was suggested by Shillitoe *et al.*⁹ who used an enzyme-linked immunosorbent assay (ELISA) and claimed that patients with primary sicca syndrome had raised levels of both IgG and IgM antibodies to CMV, indicating active infection. The IgM antibodies were thought not to be false positives due to rheumatoid factors, as the reaction persisted after absorbing the sera with protein A Sepharose. They also found that antibodies to mumps virus were not raised and suggested that their findings indicated a specific hyper-responsiveness to CMV in SS, though in this study no disease controls were used.

More recent work suggests that a potential role for EBV in SS is also worth investigating. Approximately 40% of patients with SS have antibodies to La (SS-B).¹⁰⁻¹³ This antigen is involved in proces-

sing both host and viral ribonucleic acids (RNAs),¹⁴⁻¹⁶ including EBV RNA,¹⁴ with which it forms stable complexes. It is possible that La, a host protein, may become antigenic when it forms such complexes.

In this study we compare titres of antibodies to EBVCA and CMV in patients with RA, SS, and normal controls. This approach allows each patient group to act as a disease control for the group under examination and CMV to act as a relevant specificity control for EBV and vice versa, as both are herpes viruses. By these means we examined whether there was any seroepidemiological evidence for a role for either virus in SS or RA.

Patients and methods

PATIENTS AND SERA

Normal sera were taken from 26 healthy adult volunteers (three male and 23 female) with a mean age of 42.3 years. Twenty-six patients with sicca syndrome comparable for age (mean 45.5 years) and sex (male:female 1:25) and 41 older patients with RA (mean age 60.9 years, male:female 9:38) were coded and assayed for titres of antibodies to CMV and EBV without knowledge of the diagnosis. Three of the RA sera that were examined for anti-CMV were not assayed for anti-EBVCA; otherwise all sera were examined in both assays.

Sicca syndrome was diagnosed only if all three of the following were found: (a) a complaint of dry eyes or mouth, or both; (b) a positive Schirmer's test (less than 10 mm in both eyes); and (c) rose bengal staining or positive lip biopsy, or both. Patients were excluded if they fulfilled more than three American Rheumatism Association (ARA) criteria for systemic lupus erythematosus.¹⁷ None of the patients with SS was receiving treatment with steroids or cytotoxic drugs. All the patients with RA fulfilled ARA criteria for classical or definite RA.¹⁸ Nineteen of the patients were being treated, or had been treated, with gold or penicillamine, eighteen with steroids and/or cytotoxic drugs, and five with non-steroidal anti-inflammatory agents only.

SEROLOGICAL STUDIES

Titres of IgG antibodies to EBVCA were measured by indirect immunofluorescence, with doubling dilutions of sera from an initial serum dilution of 1:8.⁶

Antibodies to CMV were measured by a solid phase radioimmunoassay, and rheumatoid factors were depleted by adsorption with IgG coated latex beads as previously described.¹⁹⁻²¹ Antibodies to La were detected by counterimmunoelectrophoresis with rabbit thymus acetone powder and their identity confirmed with precipitin lines of identity on immunodiffusion.¹³

The Wilcoxon rank sum test and χ^2 test were used for the statistical analysis of differences in titres between the different disease groups.

Results

RHEUMATOID ARTHRITIS

The log₂ geometric mean titre (GMT) of antibodies to EBVCA was virtually identical (7.66) to SS (7.45) and normal controls (7.42) (p – not significant) (Fig. 1). The titres did not correlate with the presence of extra-articular disease, including Sjögren's syndrome. Patients treated with gold or penicillamine had a higher GMT (8.35) compared with the remainder (7.05) (p – not significant, not shown). The frequency of IgG anti-CMV antibodies was higher in the RA group (68%) compared with the others (50%), but the GMT of the positive sera was the same (Fig. 2). Twenty sera were positive in the assay for IgM antibodies to CMV. Of these, 16 were abolished and four reduced by adsorption with latex IgG. Three of the four still positive after latex IgG

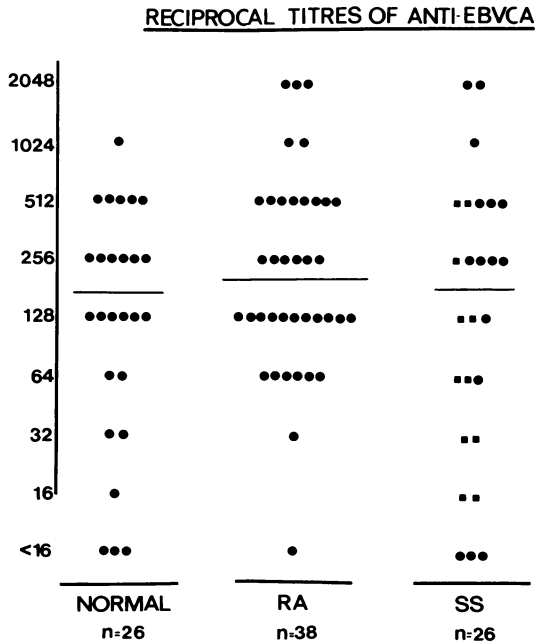


Fig. 1 Reciprocal titres of IgG antibodies to Epstein-Barr virus capsid antigen (EBVCA) in sera from patients with rheumatoid arthritis, sicca syndrome, and normal controls. The geometric mean titres of the antibody positive sera are shown as the horizontal lines. Sera from SS patients containing anti-La are shown as ■.

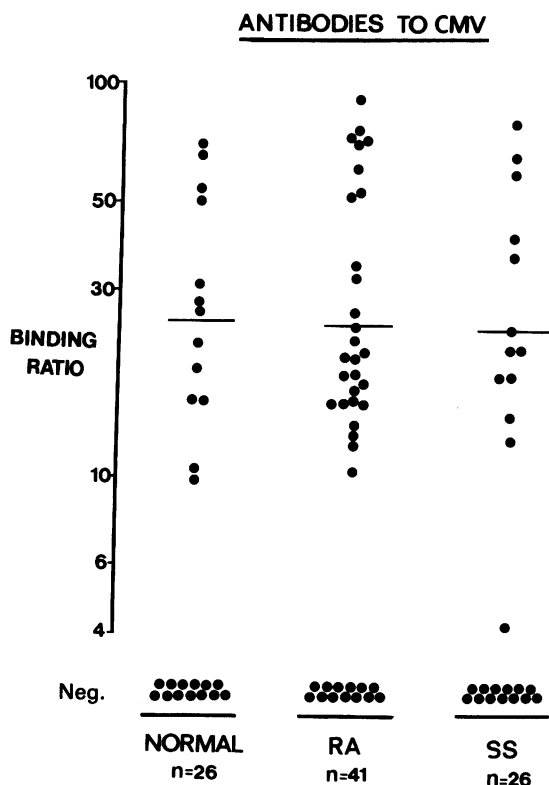


Fig. 2 IgG antibodies to CMV expressed as a test/control binding ratio in sera from patients with rheumatoid arthritis, sicca syndrome, and normal controls.

treatment were abolished with subsequent adsorptions.

SICCA SYNDROME

The frequency (50%) and GMT of IgG anti-CMV were identical in SS to those of a normal control group of similar age and sex distribution (Fig. 2), and there was no difference in titre in the sera containing anti-La (not shown). False positive IgM anti-CMV antibodies that disappeared after adsorption with latex IgG were detected in one serum. The prevalence and mean titres of anti-EBVCA were similar to those of the controls (Fig. 1). All of the sera containing anti-La contained IgG antibodies to EBVCA, though the mean \log_2 GMT tended to be lower, 6.36 (p – not significant).

Discussion

In this report the titre of antibodies to EBVCA was higher in the sera from patients with RA, though it

was marginal ($0.25 \log_2$) and in our study could be related to treatment with gold and penicillamine. Much larger numbers would be needed in our work and previous studies to confirm this and to ascertain whether the effect was directly due to the treatment or to the possibility that the gold or penicillamine treated group represented a different population of patients.

The difference in anti-EBVCA titres between RA and normal sera was trivial compared with the 6 \log_2 differences in anti-RANA titres which we have previously described.⁶ A similar dissociation between anti-EBVCA and anti-RANA titres was reported in a previous study³ where a 1 \log_2 , i.e., one doubling dilution, difference in anti-VCA titres was observed compared with an approximately 16-fold difference in anti-RANA titres. This supports the hypothesis that the increased anti-RANA titres in RA are not due to a generalised hyper-responsiveness to EBV, particularly as patients with Burkitt's lymphoma, in whom active EBV infection is marked by very high titres of anti-EBVCA and anti-EBNA, were reported as negative for anti-RANA antibodies.²² The recent finding that determinants for RANA can be detected on the EBNA polypeptide by immunoblotting^{7, 8} suggests another apparent discrepancy, as anti-EBNA titres in RA are usually found to be normal or only marginally raised,^{1, 3, 4} and we have reported RA sera negative for anti-EBNA antibodies in which anti-RANA was detected both by immunodiffusion and immunoblotting.^{6, 7} These findings can be explained by the possibility that anti-RANA antibodies react with determinants on the polypeptide that are different from those detected by anti-EBNA. Alternatively, as we have previously suggested⁷ sera from patients with RA may contain antinuclear antibodies that cross react with the EBNA polypeptide. The second mechanism is supported by the recent description²³ of a 62 000 host protein that shares antigenic components with EBNA, which may reflect similarities between part of the EBV genome and host DNA.²⁴ We suggest that the RANA-anti-RANA reaction may be more complex than previously thought, and the higher anti-RANA titres in RA may not be due to a generalised hyper-responsiveness to EBV.

The prevalence and titre of anti-CMV in SS were the same as in the normal controls. IgM antibodies, which were mainly detected in the RA group, were false positives due to rheumatoid factors. We are unable to reconcile our results with those of Shillitoe *et al.*,⁹ who found that two thirds of their patients with SS had IgM antibodies to CMV and that the mean titre of IgG antibodies was significantly higher than for normal controls. The reason for the

differences in our results is unclear and must be due to either differences in the assays or to differences in the patient population.

Unlike the ELISA reported by Shillitoe *et al.*⁹ the radioimmunoassay described in this report has been extensively examined in seroepidemiological studies.¹⁹⁻²¹ Since both EBV and CMV are herpesviruses associated with long term chronic infection, leading to a persistent antibody response, we suggest that an important criterion for a valid assay is that it should be able to show a distinction between individuals infected with the virus and those who are not. Such a distinction is clearly presented here, though it was not apparent in the data shown in the ELISA assay,⁹ where both control and SS sera showed a continuous range of values from zero to strongly positive. This suggests that serum factors other than anti-CMV may have contributed to the findings of Shillitoe *et al.*

Although only one patient with SS in our study had false positive IgM anti-CMV antibodies, the high frequency in RA suggested that it was due to rheumatoid factors acting as a bridge between IgG antibodies and the conjugate. This was confirmed by disappearance of the IgM reaction after adsorption with latex IgG. Approximately two thirds of patients with SS have rheumatoid factors, and the majority have raised serum levels of IgG. It is possible that the procedure used by Shillitoe *et al.* (absorption of IgG by protein A, which was apparently adequate for normal sera)⁹ might not have excluded interfering rheumatoid factors or removed high levels of IgG from these pathological sera. An additional factor, which makes interpretation of their data difficult, is that the sensitivity and specificity of their assay were not validated with IgM positive control sera from patients known to have active CMV infection.

An alternative explanation for our failure to confirm evidence of active CMV infection in SS is that the sera used in our study came from a different population of patients, an issue which would have been resolved by an exchange of sera with the group in the original report,⁹ though these are apparently not available for further study.

The geometric mean titre of anti-EBVCA in SS was also identical to the controls, though those sera that also contained anti-La (SS-B) tended to be lower. One reason for this low titre may be the strong background cytoplasmic immunofluorescence produced by anti-La itself, demonstrable with monoclonal antibodies tested on lymphoblastoid cell lines,²⁵ which may have masked specific VCA staining. Although this does not provide evidence against EBV as a possible aetiological agent in SS, it indicates the limitations of seroepi-

demiological techniques for investigating a viral aetiology for connective tissue diseases. These findings, together with our negative data for CMV, highlight the importance of incorporating adequate laboratory and disease controls when titres of antibodies to viruses are measured in sera containing autoantibodies and rheumatoid factors.

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Book review

Perspectives in Rheumatology 1984. Eds. John Decker and Tom Scott. Pp. 266. £30.00. Current Medical Literature: London. 1984.

This is a beautifully produced book; well bound, good quality paper, pleasing typeface, and excellent layout. It differs from other review volumes—in addition to 'standard' commissioned review articles a large selection of references from many journals has been grouped into topics and a short resumé of each set of papers has been provided by one of the associate editors. One might expect such a wide coverage to be sketchy, but the whole volume is large enough to be very comprehensive.

However, although the Preface suggests that this might be the first of a biennial venture and claims that the literature review covers the last two years, this is sadly not true. By the time the book has hit the shops—1985—no reference is later than 1983 and many date back five years or more. Though this allows a broad perspective, to be always two years behind is a fairly serious problem for a supposedly current review.

Nonetheless I enjoyed two articles; John Decker's own, very readable one on treatment in rheumatoid arthritis and Hunder and Hall's article on disease classification, referring among others to three papers on juvenile arthritis

which I had missed and will be getting my librarian to copy. Several other references in various topic groups had also passed me by. I admit to skimming the long systemic lupus erythematosus section, partly because it was so awfully boring and partly because having only seen one patient with the disease last year I couldn't really get up any feeling of need.

Now rheumatologists world wide receive a bimonthly digest from CML, circulated under sponsorship, identical in concept to this compendium, and with the same team of associate editors. Do we need the compendium as well? Frankly I don't think the format works in the large book. It is too dated and too indigestible. Whereas the bimonthly version can be quickly enjoyed, the book demands a certain dedication, like homework. I am told that it may also be distributed free, courtesy of the pharmaceutical industry and would be an elegant addition to a unit library, but I doubt if it would be opened very often. It is too specialised for most postgraduate centre libraries and in these straightened times I can think of better buys.

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