Virus antibody levels and delayed hypersensitivity in rheumatoid arthritis

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Phillips, P. E., Waxman, J., Hirshaut, Y., and Kaplan, M. H. (1976). *Annals of the Rheumatic Diseases*, 35, 152–154. Virus antibody levels and delayed hypersensitivity in rheumatoid arthritis. Epstein-Barr virus and cytomegalovirus antibody levels were not higher in patients with rheumatoid arthritis compared to matched controls. Delayed hypersensitivity, measured by skin test reactivity, was depressed in rheumatoid arthritis. There was no correlation between virus antibody titres and delayed hypersensitivity.

The hypothesis that rheumatoid arthritis (RA) is caused by an infectious agent is widely accepted. However, attempts to implicate specific agents, whether by isolation or serology, have been unsuccessful (Phillips, 1976). Cellular immunity has not previously been studied in conjunction with humoral immunity to viruses. We report here a negative serological study in RA of antibodies to two subgroup B herpesviruses, Epstein-Barr virus (EBV) and cytomegalovirus (CMV). No correlation was found between either virus antibody level and delayed hypersensitivity.

Patients and methods

Thirty-three patients with classical RA (Ropes and others, 1958) were matched for age (mean 55.7 years, range 22–74) and sex (27 females, 6 males) with each of two control groups. The other disease controls were hospitalized patients: 18 degenerative joint disease, 5 herniated nucleus pulposus, 7 miscellaneous orthopaedic problems (trauma, ganglion, bursitis), and 3 infections. The normal controls were blood donors.

Sera were stored at -20°C and coded before testing for EBV antibody by indirect immunofluorescence (Hirshaut and others, 1970) and CMV antibody by microcomplement fixation (Armstrong and others, 1971). Titres were expressed as the $\log_2$ of the reciprocal of the highest positive serum dilution.

Delayed hypersensitivity was measured by intradermal testing with 5 antigens (tuberculin, mumps, streptokinase-streptodornase, trichophyton, and candida) as described

previously (Waxman and others, 1973). Induration was measured in mm at 48 hours, and the sum of these measurements defined as the skin test index.

Student's 't' test was used to measure the significance of differences between means, and Pearson's correlation coefficient for relationships between two variables (Croxtan, 1959).

Results

The incidence and distribution of EBV antibody titres were similar in RA and both control groups. Two RA patients and one normal control lacked antibody. Mean EBV antibody was similar in RA to both control groups (Table), although the other disease controls were significantly higher than the normal controls.

The incidence and distribution of CMV antibody was also similar in RA and the other disease control group; the normal controls were not tested. Eight RA patients and 6 controls lacked antibody. Mean CMV antibody was similar in both groups (Table). Additional unmatched classical RA patients were also tested for CMV antibody; the mean titre (3.96) for the entire group of 55 RA patients was similar to the matched subgroup and still not different from the controls.

Delayed hypersensitivity, as measured by skin testing, was significantly reduced in RA patients compared to the other disease controls (Table); the normal controls were not tested.

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Table Mean levels of Epstein-Barr virus (EBV) and cytomegalovirus (CMV) antibodies and skin test index in RA and controls

	Rheumatoid arthritis	Controls	Normal
		Other disease	
EBV antibody (log ₂)			
Mean	8.33	9.06*	7.89
SD	2.44	1.56	1.64
Range	Neg-13.3	7.3-13.3	Neg-11.3
CMV antibody (log ₂)			
Mean	4.21	4.32	NT
SD	2.11	1.94	
Range	Neg-8	Neg-6	
Skin test index			
Mean	7.3†	23.2	NT
SD	10.5	18.7	
Range	0-30	0-57	

* Significantly higher than normal controls, $P < 0.005$.† Significantly lower than controls, $P < 0.01$.

NT = not tested.

Significant correlations were not found between age, sex, or skin test index and either virus antibody titre, nor between the two virus antibodies themselves. The only significant correlation was an inverse one between skin test index and age in the RA group ($r = -0.38$; $P < 0.05$).

Discussion

The normal levels of EBV and CMV antibody do not support an aetiological role for either of these viruses in RA. Other virus antibodies have also been studied in RA, but no consistent increase in titre or incidence has emerged (Phillips, 1976). The role of viruses in RA thus remains speculative.

The decreased skin reactivity in RA has been described previously, and these patients were part of that study (Waxman and others, 1973). The decreasing skin reactivity with older age found here no doubt reflects the decreasing reactivity associated with longer disease duration found there. Treatment did not affect skin reactivity; aspirin and corticosteroid (maximum daily dose 10 mg prednisone) usage were similar in both high and low reactivity RA groups.

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It is probable that the low reactivity found overall in RA is not specific for this disease. Rather it may indicate 'preoccupation' of the lymphocyte response with a foreign antigen. Other examples of this may be the decreased skin reactivity found during acute viral infections, lepromatous leprosy, and sarcoidosis (Waxman and others, 1973).

Correlations between humoral immunity to viruses and cellular immunity as measured by skin testing have not previously been sought in RA. However, in lepromatous leprosy where skin reactivity is reduced, high EBV antibodies were found, while in the tuberculoid form where skin reactivity is intact or enhanced, EBV antibody was normal (Papageorgiou and others, 1971). Those authors postulated that the decreased cellular immunity in the lepromatous type allowed enhanced EBV replication, thus stimulating more antibody production. By extrapolation, they hypothesized a similar reason for the high EBV antibodies in sarcoidosis, where skin reactivity is also reduced, and further that high EBV antibodies would be found in other diseases with impaired cellular immunity (Papageorgiou and others, 1971). In this study no such relationship was found, suggesting the lack of any such general linkage between the two types of immune response, at least to the various microbial antigens used here.

It might be expected that increased specific humoral immunity with either absent or increased *specific* cellular immunity would be found to the postulated infectious agent causing RA (Phillips, 1976). Studies of both types of immune response to the *same* microbial agent have not been reported. EBV and CMV skin test antigens are not available, thus assessment of specific cellular immunity to these agents would require *in vitro* testing. However, the lack of increased antibodies to EBV and CMV in the presence of a depressed cellular response to other microbial, including virus, antigens suggests that no specific abnormalities of the cellular response to EBV and CMV would be found.

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