Antibody producing capacity to the bacteriophage $\phi X174$ in rheumatoid arthritis

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SUMMARY A study of antibody production in response to a primary immunogen, the bacteriophage $\phi X174$, was performed in 27 patients with rheumatoid arthritis and 15 controls. All patients produced a primary (IgM) response to initial immunisation. The frequency distribution of peak antibody titres after secondary immunisation showed a marked difference between the patients and controls, with 10 patients having peak titres below 5000. The IgG component of the antibody response expressed as a percentage of total phage antibody on the 10th day after secondary immunisation was less in the patients than in the control group. There was no correlation between antibody titres and indices of disease activity, rheumatoid factor titres, or the presence of DRw4, DRw3, and DRw2. After secondary immunisation the patients with rheumatoid arthritis were treated with d-penicillamine, azathioprine, levamisole, or maintained on a non-steroidal anti-inflammatory drug. Assessment of response to tertiary immunisation again showed an impairment of antibody production in the rheumatoid group receiving non-steroidal anti-inflammatory drugs compared with the controls. None of the drugs, d-penicillamine, azathioprine, or levamisole, produced further suppression or augmentation of antibody production in response to the immunogen.

Key words: immunosuppressive therapy.

Rheumatoid arthritis is characterised by a chronic synovitis and the production of IgM and IgG antitoglobulins. Panayi et al have shown a high prevalence of the B cell isoantigen DRw4 in patients with this disease and have also shown that DRw3 and DRw2 are markers for high and low titres of autoantibodies respectively.1 Rheumatoid disease could develop as the result of an inappropriate immune response to an extrinsic antigen in individuals with a particular genetic background as shown by the presence of DRw4. The clinical expression of disease and the intensity of autoantibody production could be under the influence of DRw3 and DRw2. The object of this study was to assess the integrity of the antibody producing system in response to immunisation with a primary immunogen the bacteriophage $\phi X174$. Primary and secondary immunisation procedures were performed and both the quality and class of antibody produced were measured. Patients with rheumatoid arthritis were treated with either d-penicillamine, azathioprine, or levamisole, and their response to further immunisation was compared with that of controls.

Patients and methods

Patients
Twenty seven patients with rheumatoid arthritis were studied with ages ranging from 41 to 68 years (mean 57.4). Duration of their disease ranged from six months to 22 years with a mean of 7.1 years. All patients had a generalised active synovitis at the time of the study and this was measured objectively by means of a computer linked infrared thermography according to the method of Ring et al.2 An x ray examination showed a total of 19 patients with evidence of articular erosions. Six patients had
subcutaneous nodules and seven patients other extra-articular features, namely pulmonary involvement in four patients, scleritis in one patient, splenomegaly in one patient, and Felty's syndrome in one patient.

A total of 15 controls (seven male, eight female) was studied (age range 29-75 years, mean 48.4). Five of these controls had evidence of osteoarthritis.

During the period of study involving primary and secondary immunisation all patients were maintained on non-steroidal anti-inflammatory drugs. After secondary immunisation six patients continued to receive non-steroidal anti-inflammatory drugs. Seven received D-penicillamine in a dose of 125 mg daily for two weeks, increasing by 125 mg daily increments every two weeks until a maximum

![Fig. 1 Antibody titres in response to primary immunisation in normal controls. One control only attended for blood sampling on day 28 after immunisation (Ab titre 7500).]

![Fig. 2 Antibody titres in response to primary immunisation in patients with rheumatoid arthritis.]

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A dose of 500 mg of d-penicillamine daily was administered. Seven patients were treated with azathioprine 150 mg daily and seven patients received levamisole in a dose of 150 mg on Tuesdays and Thursdays. These drugs were continued for a period of four months and during the fourth month the response to tertiary immunisation with the bacteriophage was assessed.

RHEUMATOID FACTOR
Rheumatoid factors were measured in the serum by radioimmunooassay. IgM rheumatoid factor was measured by a modified method of Hay et al. and IgG rheumatoid factor using baboon IgG as antigen and radiolabelled baboon antihuman IgG to measure the uptake of IgG rheumatoid factor.

IMMUNISATION PROCEDURE
The bacteriophage $\phi$X174 was prepared in the laboratory and checked for sterility and pyrogens before human administration. Patients and controls were given the antigen intravenously in a dose of $5 \times 10^9$ plaque forming units per injection with an interval of one month between the primary and secondary immunisation. All patients and nine controls were given a third injection of antigen in the same dose three months after secondary immunisation. Venous blood samples were collected before immunisation and on days 3, 7, 10, 14, 21, and 28 after primary, secondary, and tertiary immunisation.

ANTIBODY ASSAY
Total antibody in each serum sample was measured by a plaque assay method. The presence of pre-existing antibody to the bacteriophage was excluded by examination of serum collected before primary immunisation. Confirmation of a true primary (IgM) response after initial immunisation was carried out by zonal centrifugation of serum on a sucrose density gradient. Dialysed fractions were tested for (a) antibody activity by bacteriophage neutralisation and (b) the presence of IgM and IgG by imunoelectrophoresis and radial immunodiffusion.

Serum collected on the 10th day after secondary immunisation was examined by the plaque assay method before and after treatment with dithiothreitol. This reagent inactivates the IgM component of $\phi$X174 antibody. The method described by Pirofsky and Rosner was used.

HLA TYPING
Peripheral blood lymphocytes from the patients were typed for HLA at the Sheffield blood transfusion service. The standard microcytotoxicity test was performed.

Fig. 3 Antibody titres in response to secondary immunisation in normal controls.
Results

The results of antibody titres after primary and secondary immunisation are shown in Figs 1 and 2. There was no pre-existing antibody in the sera collected before primary immunisation. After the first immunisation all patients produced antibody and this was entirely of the IgM class, confirming a true primary response. There was wide variation in rate of production of antibody and peak titres both in patients and controls. Some patients showed low peak titres, but as a group these were not significantly different from those of the controls.

The results of antibody titres after secondary immunisation are shown in Figs 3 and 4. There was a marked variation in total antibody produced in the rheumatoid group compared with the controls. Analysis of the frequency distribution in the two groups demonstrated that a total of 10 patients showed impairment of antibody production with peak titres of less than 5000. Some individuals showed minimal amplification of pre-existing antibody levels.

In addition to overall impairment of antibody production, there was also a defect in conversion to IgG class of antibody after secondary immunisation (Fig. 5). There was no correlation between the total antibody and IgG component antibody titres. One individual showed a very high peak total antibody response of 90,000, but no IgG component was detected in the serum. The mean proportion of IgG expressed as a percentage of total antibody on day 10 after secondary immunisation was significantly less in the rheumatoid (8%) than in the control group (17%) (p<0.01).

Analysis of the results of total antibody production in terms of peak titres after secondary immunisation showed no correlation with disease duration or activity. Fig. 6 shows the relation between disease activity in terms of the thrombogenic index and peak antibody titres; there was no correlation (p>0.5). In addition, there was no correlation between peak antibody titres and either IgM (Fig. 7) or IgG (Fig. 8) rheumatoid factor titres.

The results of HLA typing are shown in Table 1. A total of 26 patients with rheumatoid arthritis were investigated. The prevalence of HLA-DRw4, DRw3, DRw2 in the rheumatoid group was compared with the prevalence in a total of 384 normal blood donors. DRw4 occurred in 22 of 26 of the rheumatoid patients (84%) compared with 130 of 384 of the controls (33.9%) (p<0.001). DRw3 occurred in only two patients (7.7%) and in 105 (27.3%) of the controls (p<0.05) and DRw2 in four patients (15.4%) and in 133 (34.6%) of the controls (p<0.05). HLA-DRw4, DRw3, and DRw2 when present were not clearly associated with either augmented or suppressed antibody production. In
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Fig. 5 IgG component of antibody response on day 10 after secondary immunisation with φX174 in patients with rheumatoid arthritis and in normal controls.

Fig. 6 Peak antibody titre after secondary immunisation of rheumatoid arthritis patients with φX174 plotted against activity of inflammatory joint disease assessed using a thermographic index.

Fig. 7 Peak antibody titres after secondary immunisation with φX174 plotted against IgM rheumatoid factor titres.

Fig. 8 Peak antibody titres after secondary immunisation with φX174 plotted against IgG rheumatoid factor titres.
sufficient numbers of control subjects were tissue typed to allow any assessment of possible relationships between the magnitude of immune response to φX174 and DRw4, DRw3, and DRw2.

There was no relation between these serotypes and the integrity of the mechanism for conversion from IgM to IgG production after secondary immunisation in the rheumatoid group.

The results of antibody production after tertiary immunisation are shown in Figs 9 and 10. The nine normal controls studied all showed peak antibody titres exceeding 20 000. In the rheumatoid group a similar depression of response was shown as following secondary immunisation—seven patients showing peak titres less than 20 000 and three patients showing very low titres of less than 1000. Analysis of the results for the three drug groups and controls showed no evidence for an overall suppression or augmentation of antibody production. The mean increases in antibody titres between the peak secondary and tertiary responses are shown in Table 2.

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**Table 1 HLA-DRw typing**

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<th>DRw4</th>
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<th>DRw2</th>
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<td>22</td>
<td>2</td>
<td>4</td>
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<td><strong>Controls (blood donors), n (%)</strong></td>
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<td>130</td>
<td>105</td>
<td>133</td>
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<td><strong>RA/control r &amp; p Value</strong></td>
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**Fig. 9** Antibody titres in response to tertiary immunisation with φX174 in normal controls.
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Discussion

Previous studies of antibody producing capacity in rheumatoid arthritis have produced conflicting results. Three groups have studied the primary response with brucella as antigen. One found increased responses,$^7$ whereas the others obtained responses within the normal range.$^8^9$ With tetanus toxoid as antigen Greenwood and Barr found an augmented primary response.$^{10}$ In contrast, Vaughan and Butler showed a normal secondary response$^{11}$ and Whaley et al a depressed secondary response.$^{12}$ Finally, Hazleman and Currey found a normal primary response on immunisation with typhoid.$^{13}$ The disadvantages of the previous studies are threefold. Firstly, the antigens used cannot be guaranteed as primary immunogens as either natural exposure or immunisation may have occurred. Secondly, antigens were administered via either the intramuscular or subcutaneous routes, and this may result in variation in rates of absorption and mode of presentation to the immune system. Thirdly, either the primary or the secondary response was measured and not both.

The antigen used in this study was the bacteriophage $\phi$X174, which is an established primary immunogen. It is a single stranded DNA virus infective for the C strain of Escherichia coli. It is administered by the intravenous route, and antibody is measured by a plaque assay method. Previous studies have shown that the bacteriophage $\phi$X174 is a safe and powerful primary immunogen that can be used for assessment of antibody producing capacity in a wide range of diseases.$^5^7^{14}^{15}$ Both total antibody and IgM and IgG components of antibody produced can be measured.
Using this technique for studying antibody producing capacity in patients with rheumatoid arthritis, we have shown that a subpopulation of these individuals had an impaired ability to mount an immune response. This defect relates both to total antibody production and the IgG component of antibody produced after secondary immunisation.

The defects in antibody production could represent either a primary B cell defect or be secondary to disordered helper or suppressor T cell control. This could only be proved if the immune system were to be studied before onset of the rheumatoid process. In a separate study we have had the opportunity of assessing antibody producing capacity to φX174 in a group of patients who had previously shown features of arthritis related to infection with Yersinia enterocolitica. These individuals showed no overt evidence of disease at the time of the study. There was no significant difference between antibody production in these individuals and a control group.

It is possible that the defects in antibody production shown in the patients with rheumatoid arthritis are a secondary phenomenon. For example, it is known that a primary immune response can be suppressed if an immune response to another antigen is present at the same time (antigen competition). A relation has been shown between persisting circulating immune complexes and a reduction in the response to sheep erythrocytes in mice. This has prompted the suggestion that chronic circulating immune complexes may cause non-antigen specific immunodepression. In this study there was no correlation between antibody producing capacity and the presence of hypocomplementaemia and circulating immune complexes as detected by the presence of anticomplementary activity and the ability of sera to aggregate platelets. Furthermore, as rheumatoid synovitis can be viewed as a form of extravascular immune complex disease one might expect to see an inverse relation between the intensity of rheumatoid synovitis and the degree of immunosuppression. There was no evidence for such a relation as judged by clinical assessment and thermographic indices of joint inflammation.

Alternatively, the immunosuppression seen in this study could be explained in terms of antigenic competition with an autoantigen such as IgG. This is rendered unlikely by the fact that there was no relation between either IgM or IgG rheumatoid factor titres and immunosuppression. Finally, the impaired response could be due to antigenic competition with a persisting microbial antigen. This is suggested by the recent demonstration by Johnson and his colleagues that there is increased systemic immunisation by bacterial components in rheumatoid arthritis. If antigenic competition is causing the observed immunosuppression, however, it is difficult to explain why it affected the secondary and tertiary response to immunisation while leaving the primary antibody response apparently unimpaired. Thus the question as to whether the abnormalities in antibody production demonstrated represent a primary abnormality in the immune system in these individuals, thereby predisposing them to rheumatoid disease, or whether they represent a phenomenon secondary to another immune mediated chronic inflammatory disease must remain open to debate. If the defects are primary there is no evidence from this study that they are under genetic control related to the isoantigens DRw4, DRw3, and DRw2. At the time this study took place, however, an insufficient number of control subjects were typed for DRw4, DRw3, and DRw2. It is not possible, therefore, to draw any conclusion regarding the possible relation between these serotypes and the magnitude of antibody responses after immunisation with a primary immunogen.

The terms immunosuppression and immunostimulation are used to describe the effects of drugs such as d-penicillamine, azathioprine, and levamisole on rheumatoid arthritis. Hunneyball et al. showed that rabbits treated with long term oral doses of d-penicillamine showed a depressed antibody response in vitro to immunisation with egg albumin. Lipsky and Ziff showed, using in vitro studies, that d-penicillamine may act as a selective inhibitor of helper T cell function. Our results do not show any evidence either for augmentation or suppression of antibody production. Similarly, azathioprine does not suppress a patient's ability to mount an adequate antibody response to a primary immunogen. In fact, of four patients who showed impaired responses on secondary immunisation, three of these produced normal peak responses to tertiary immunisation while receiving azathioprine, suggesting an augmented response. Levamisole has been shown to be effective in the treatment of rheumatoid arthritis, but its mechanism is unclear. Its potential for restoration of impaired cell mediated immunity may be relevant. Our data do not indicate either augmentation or suppression of antibody production. Two patients who showed marked impairment of antibody production on secondary immunisation again failed to augment their responses after tertiary immunisation while receiving levamisole.

In conclusion, a defect in antibody production in terms of both total antibody and conversion from IgM to IgG antibody after secondary immunisation has been shown in a subpopulation of patients with
rheumatoid arthritis. The mechanism for these defects, however, remains unclear.

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References