Antibody producing capacity to the bacteriophage ϕ X174 in yersinia arthritis

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SUMMARY Antibody production in response to the primary immunogen bacteriophage ϕ X174 was investigated in 14 patients with previous yersinia arthritis (YA) and in 15 controls. HLA-B27 occurred in 10 patients with YA and in three controls. After primary and secondary immunisation the antibody responses were essentially similar both in patients with YA and controls. Consequently our results suggest that antibody response to a foreign antigen does not differ between patients with YA and a normal control population. In addition, there was no difference in peak antibody responses between individuals with HLA-B27 and those without HLA-B27.

Key words: *Yersinia enterocolitica*, HLA-B27, immunisation.

Yersinia enterocolitica infection may be followed by inflammation involving the synovium, skin, urogenital tract, and heart. This results in the clinical features of arthritis, erythema nodosum, carditis, and signs of urological involvement. Yersinia arthritis (YA) is highly associated with the presence of HLA-B27.^{1–4} Extra-articular manifestations such as ocular inflammation, carditis, and signs of urological inflammation are frequent,⁵ and the duration of the acute arthritis is longer in B27 positive patients with YA, whereas erythema nodosum seems to be more frequent in B27 negative patients.⁶

The pathogenesis of the B27 associated diseases, such as YA, Reiter's disease, and ankylosing spondylitis, is obscure. In addition to the clinical findings above, which may be immunologically mediated, there is some evidence for the influence of B27 on some immunological and leucocyte functions. Lymphocyte transformation tests or skin testing using various antigens have given normal, depressed, or enhanced responses in patients with ankylosing spondylitis or in their B27 positive or negative relatives.^{7–11} Mixed lymphocyte reaction is depressed in patients with ankylosing spondylitis,

and also in their B27 positive relatives compared with B27 negative relatives.^{7 12 13} Less is known about humoral immune response in B27 positive individuals. Streptococcal antibodies are higher in B27 positive than in B27 negative relatives of patients with ankylosing spondylitis,¹⁰ whereas humoral immunity against tetanus toxoid and salmonella O antigen is normal in patients with ankylosing spondylitis.⁹ Neutrophil chemotactic response is higher in B27 positive individuals.¹⁴

The object of this study was to assess the antibody producing capacity in patients with previous YA using an antigen against which a humoral immune response has not usually been found to occur. Primary and secondary immunisation procedures were carried out and particular attention was focused on the secondary response in terms of both total antibody and the IgG component of antibody at the time of the peak response. The results were analysed considering both the clinical pattern of disease and the presence or absence of HLA-B27.

Patients and methods

A total of 14 Finnish patients who had had YA were investigated after complete recovery. The diagnosis had been established by the demonstration of a typical clinical picture¹⁵ associated with changing titres of antibodies to *Y enterocolitica* serotypes 3 or

Table 1 Clinical characteristics of patients and controls

	Patients	Controls
Number	14	15
Female/male ratio	3/11	8/7
Mean age (years)	43.8	32.6
Age range (years)	(30-65)	(23-44)
HLA-B27 positive	10	3

9. Stool cultures for *Y enterocolitica* were studied in nine patients, with positive culture in one of the patients. At the time of the study no patient showed evidence of persisting synovitis, but five patients had evidence of axial joint involvement, including one with ankylosing spondylitis present before YA and two with radiological sacroiliitis. Two patients had previously recorded episodes of iritis. Three patients were receiving non-steroidal anti-inflammatory drugs for axial joint involvement at the time of the study. The control group consisted of 15 normal individuals (10 English, five Finnish). The antibody titres against *Y enterocolitica* were below the lower limit of normal value in all the controls.

Peripheral blood lymphocytes from the patients were typed for HLA either at the Finnish Red Cross blood transfusion service in Helsinki or at the Sheffield regional blood transfusion service. The standard NIH two stage microcytotoxicity test was performed in both laboratories. The immunisation

procedure was performed as follows: Two doses of 5×10^9 plaque forming units of the bacteriophage $\phi X174$ were administered intravenously with a 28 day interval between the two immunisations. Venous blood samples were collected before primary immunisation, on days 3, 7, 14, 21, and 28 after this injection, and on days 3, 7, 10, 14, 21, and 28 after secondary immunisation. A further serum sample was collected one year after secondary immunisation.

Total antibody in each serum sample was measured by means of the plaque assay method described by Peacock *et al.*¹⁶ Confirmation of a true primary response by the demonstration of IgM antibody after primary immunisation was shown by subjecting serum to rate zone centrifugation on a sucrose density gradient. Fractions (0.5 ml) were tested for (a) antibody activity by bacteriophage neutralisation and (b) presence of IgG and IgM by immunoelectrophoresis and radial immunodiffusion. Serum samples collected on the 10th day after secondary immunisation were tested for antibody before and after treatment with dithiothreitol by the method of Pirofsky and Rosner.¹⁷ This reagent inactivates the IgM component of the ϕX antibody and gives a rapid assessment of the concentration of the IgG component. Statistical significance between the peak antibody titres in the patients with YA and in the control group were calculated by Wilcoxon's two sample rank test.

Fig. 1 Serial antibody titres in response to primary immunisation with $\phi X174$ in normal controls. One control only attended for blood sampling on day 28 after immunisation (Ab titre=7500).

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Fig. 2 Serial antibody titres in response to primary immunisation with $\phi X174$ in patients with yersinia arthritis.

Fig. 3 Serial antibody titres in response to secondary immunisation with $\phi X174$ in normal controls.

Fig. 4 Serial antibody titres in response to secondary immunisation with ϕ X174 in patients with yersinia arthritis.

Results

Details of the patients and controls are shown in Table 1. Figs 1–4 show the antibody responses after primary and secondary immunisation in the patients and controls.

A low titre of pre-existing antibody was shown in one patient with YA before primary immunisation. The antibody produced, however, was entirely IgM antibody. There was no statistically significant difference in the antibody responses after primary

immunisation between the patients with YA and the control group. After secondary immunisation all the patients with YA had a peak titre exceeding 10 000, whereas four of the controls had a lower response ($p=0.057$) (Table 2). Patients who were positive for B27 showed somewhat higher peak antibody responses (mean 41 130) than those who were B27 negative (mean 33 750), but the difference was not statistically significant. There was no difference in antibody responses between those who had axial joint involvement or extra-articular features of disease, or both. Antibody detected in sera collected one year after secondary immunisation showed mean titres of 5397 in the YA group compared with 2800 in the controls. The mean titre in the B27 positive group was 4043 compared with 6750 in the B27 negative group. None of these results showed significance on statistical analysis. The proportion of IgG expressed as a percentage of total antibody to ϕ X174 in sera collected on the 10th day after secondary immunisation had a mean value of 11% in the patients with YA compared with 17% in the controls. Again this was not a significant difference. The absolute IgG antibody titres are shown in Fig. 5.

Table 2 Peak antibody titres after secondary immunisation in 14 patients with previous yersinia arthritis and in 15 controls*

	Patients	Controls
Mean	38 964	28 435
SEM	5 604	5 571
Titres <10 000	0†	4
Titres >10 000	14	11

*Analysis of peak antibody responses for patients and controls using Wilcoxon's two sample rank test showed no statistically significant difference.

†Number of subjects.

Fig. 5 The IgG component of antibody response on day 10 after secondary immunisation with ϕ X174 in patients with yersinia arthritis and in normal controls.

Discussion

The mechanism whereby the organism *Y enterocolitica* may induce a reactive arthritis in individuals is unknown. The high prevalence of HLA-B27 in these patients, however, indicates that genetic factors are important, and the susceptibility to the disease may be mediated by some until now unknown immunological mechanisms. Previous data indicate that a hyperimmune state may exist in these individuals as shown by the presence of high isohaemagglutinin titres, increased gammaglobulin concentrations, and a high prevalence of auto-antibodies.¹⁸ An increased prevalence of anti-nuclear, smooth muscle, and thyroid epithelial antibodies has been demonstrated. A prolonged and enhanced specific IgA and IgG antibody response in

patients with yersinia arthritis has also been observed by some groups¹⁹ but not by others.²⁰ Our results imply that although the immune response of patients with YA to a primary immunogen after the acute phase of the disease was slightly enhanced, the difference from that observed in control subjects, was not statistically significant. The ability to convert from IgM to IgG antibody on secondary immunisation was also normal in patients with previous YA. Furthermore, the presence of HLA-B27 both in patients and controls was not shown to be a marker for augmented antibody production. The normal antibody response to a bacteriophage administered intravenously, against which there has not previously occurred immunisation, indicates that at least some antigen processing functions are normal in patients with susceptibility to yersinia arthritis, though we did not investigate the role of mucosal immunity in the development of this condition.

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