

Depressed lymphocyte transformation by yersinia and *Escherichia coli* in yersinia arthritis

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SUMMARY Lymphocyte transformation responses were studied in 21 patients with acute yersinia infection followed-up after the acute infection for up to one year. Eight patients had reactive arthritis caused by yersinia. The responses to the causative serotype of yersinia were significantly lower ($p < 0.05$) in patients with arthritis than in those without arthritis. Stimulation with *Escherichia coli* gave lower responses than with yersinia, but with *E. coli* the difference between arthritic and nonarthritic groups was more significant ($p < 0.02$). The responses to yersinia and *E. coli* were not correlated with the presence of HLA B27. Lymphocyte transformation by purified protein derivative of tuberculin, streptokinase-streptodornase, phytohaemagglutinin, or concanavalin-A revealed no significant differences between the arthritic and nonarthritic groups. The role of the enterobacterial common antigen in the pathogenesis of reactive arthritis and ankylosing spondylitis is discussed.

Invasive strains of *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* can cause human disease.¹⁻⁴ The clinical picture is variable, and host-dependent factors may influence the development of the different symptoms. Postinfectious, nonpurulent arthritis is a common complication of yersiniosis,^{5,6} known to be strongly associated with HLA B27.⁷ Patients with this antigen more often also have other extraintestinal manifestations than HLA B27 negative patients.⁸ The major histocompatibility complex is known to control several cell-mediated immune functions.⁹ On the other hand immunity to intracellularly invasive agents such as viruses and listeria¹⁰ is mainly cell-mediated. *Yersinia* is also an intracellularly invasive organism. In fact it has been shown that lymphocytes develop a strong proliferative response to the yersinia antigen during yersiniosis.¹¹ This all suggests that cell-mediated immunity may play an important role in the pathogenesis of reactive arthritis.

At present there are no available systematic studies on cell-mediated immunity in patients developing reactive arthritis after yersinia infection.

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The disease is an interesting model for the pathogenesis of reactive arthritis, since the aetiological agent and the clinical picture are already known.

In the present work we have studied the lymphocyte transformation responses of patients with yersinia infection, either developing arthritis or not, from the acute phase of the infection up to one year, using yersinia, *Escherichia coli*, purified protein derivative of tuberculin (PPD), streptokinase-streptodornase (SK-SD), phytohaemagglutinin (PHA), and concanavalin-A (con-A) as mitogens. The results suggest that patients with yersinia arthritis have lower responses to the causative serotype of yersinia than patients without arthritis; the responses are not correlated with the presence or absence of the HLA B27 antigen.

Materials and methods

PATIENTS

The material consisted of 21 patients with symptoms compatible with a recent yersinia infection. The diagnosis was confirmed by significantly elevated antibody titres against *Yersinia enterocolitica* serotypes 3 or 9 or *Yersinia pseudotuberculosis* IA, with at least a 4-fold change in the agglutinin titres. Eighteen

patients had *Y. enterocolitica* 3, 2 had *Y. enterocolitica* 9, and one had *Y. pseudotuberculosis* IA infection. Some clinical and laboratory findings of the patients are presented in Table 1. One patient had a mild Reiter's syndrome. In the nonarthritic group the gastrointestinal symptoms were prominent. Two patients in the nonarthritic group had erythema nodosum. All patients, except one arthritic patient with lumbar pain, recovered fully within the follow-up period. Twenty-two healthy volunteers served as controls. They were all negative when tested for yersinia agglutinins.

In the acute phase 18 patients had received tetracyclines and one ampicillin; 2 patients without arthritis had received no drugs. Six patients with arthritis had taken indomethacin, one naproxen, and one toltenamid acid. During the period of the 1½–3 months after the onset of the infection 2 patients with arthritis had taken prednisone 10 mg every second day. The lymphocyte transformation tests were performed on days without prednisone. During the 3–12 months after the onset of the infection none of the patients was under any medication.

METHODS

Antigens. The strains used as antigens included the *Yersinia enterocolitica* serotype 0:3 (M.Y. O; S. Winblad), *Y. enterocolitica* 0:9 (M.Y. 79; B. Nilehn), *Y. pseudotuberculosis* IA (strain 2; W. Knapp), and *Escherichia coli* 0:111 (International Escherichia and Klebsiella Centre, Statens Seruminstitut, Copenhagen, Denmark). *Y. enterocolitica* 3 and 9 were cultivated on tryptose agar plates and *Y. pseudotuberculosis* on agar plates for 48 hours at 20°C. *E. coli* was cultivated on agar plates for 48 hours at 37°C. The resulting growth was suspended in saline, harvested by centrifugation, and washed twice in saline. The heat-killed bacteria were prepared by

heating the bacteria at 100°C for 2 hours. The bacteria were then harvested and washed. The bacteria were stored at –20°C in a concentration of 10¹¹ bacteria/ml in saline. In lymphocyte cultures bacteria were used as antigens in final concentrations of 10⁹, 10⁸, and 10⁷ bacteria/ml. PPD (Statens Seruminstitut, Copenhagen, Denmark) was used in final concentrations of 100, 1, and 0.01 µg/ml. SK-SD (Varidase, Lederle) was used in final concentrations of 100, 10, and 1 IU/ml of streptokinase and 25, 2.5 and 0.25 IU/ml of streptodornase, respectively.

Lectins. PHA-M (Difco) was used in final concentrations of 625, 125, and 25 µl/ml. Con-A (Pharmacia) was used in final concentrations of 100, 25, and 5 µg/ml.

Lymphocyte cultures. A microculture using washed blood cells based on a whole-blood micro-method^{11, 12} was used. 3 ml of venous blood with 30 µl of heparin (Medica, Finland) was centrifuged and the plasma was removed. Thereafter the blood cells were washed twice with Roswell Park Memorial Institute (RPMI) 1640 (GIBCO, New York) containing 20 000 units/ml of penicillin and 20 000 µg/ml of streptomycin. After being washed the blood cells were suspended in 1.8 ml of heat-inactivated pooled AB serum (Red Cross Blood Transfusion Centre, Helsinki, Finland) and then in 24 ml of RPMI 1640. 25 µl of antigen or mitogen solution and 225 µl of blood cell suspension was distributed into each well of a microtitre plate (Sterilin Microtiter System). All cultures were set up in triplicate and incubated at 37°C in 5% CO₂ and humidified air with yersinia or *E. coli* antigens for 114 hours. Lectin-stimulated cultures were incubated for 90 hours, and PPD- and SK-SD-stimulated cultures for 114 hours. To measure lymphocyte transformation 20 µl (0.25 µCi) of ¹²⁵I-5-iodo-2'-deoxyuridine (¹²⁵UdR; specific activity 90–110 mCi/mg; Radiochemical Centre, Amersham, England) was added together with 5-fluoro-2'-deoxyuridine (FUdR; Fluka, Buchs, Switzerland; final concentration 10⁶ M) into each well 18 hours before harvesting. The cultures were harvested with a multiple cell culture harvester (Skatron, Lierbyen, Norway) and the radioactivities of the filters were measured by an automatic well-type gamma counter (LKB-Wallac, Turku, Finland).

Statistical analysis. The statistical significance of the differences in the lymphocyte transformation response between patients with and without arthritis, and between patients with and without HLA B27 was evaluated by the Mann-Whitney U test.

Results

The results are given as the maximal responses, defined as the highest value obtained by any of the

Table 1 Some characteristics of the patients during an acute yersinia infection

	Without arthritis	With arthritis
Total	13	8
Men/women	5/8	5/3
Age (yr): mean (range)	23 (8–34)	30 (17–46)
Highest titre of yersinia agglutinins; mean (range)	3840 (640–10 240)	1680 (640–2560)
Highest ESR (mm/h); mean (range)	43 (9–101)	86 (18–126)
HLA B27 +/-	2/11	6/2
Duration of symptoms before the first transformation test (days); mean (range)	30 (14–43)	22 (13–45)

3 varying antigen or mitogen concentrations. The maximal responses were in all cases obtained with either one of the 2 highest antigen or mitogen concentrations. The responses were also calculated by different antigen and mitogen concentrations. The differences of the responses between arthritic and nonarthritic groups were as in the maximal responses (data not shown).

LYMPHOCYTE TRANSFORMATION RESPONSE TO YERSINIA ANTIGEN

The main interest in this study was focused on the possible differences in the cell-mediated immune response of patients with or without arthritis. The response in each case was tested with the same serotype of yersinia which had caused the infection. Fig. 1 shows the responses to yersinia antigen in the 2 patient groups. The presence or absence of HLA B27 is recorded marked for each patient.

The responses were highest in the acute phase and declined during the one-year follow-up period. The mean responses of patients with arthritis were significantly ($p < 0.05$) lower than those of patients without

arthritis in the tests carried out $< 1\frac{1}{2}$, $1\frac{1}{2}$ -3, and 3-6 months after onset of the infection. The difference was most clear in the first samples, where the mean response value of patients without arthritis was 2510 counts per minute (cpm) and that of arthritic patients 1230 cpm. After 6 months there was no significant difference between these 2 groups. The mean values were then 320 cpm and 280 cpm respectively.

LYMPHOCYTE TRANSFORMATION RESPONSE TO E. COLI, SK-SD AND PPD

Fig. 2 shows that the *E. coli* antigen also stimulated a response, which was generally less than that against the yersinia antigen but otherwise mimicked the responses to yersinia. The mean response of patients without arthritis was 1670 cpm and that of patients with arthritis 290 cpm ($p < 0.02$) in the test at $< 1\frac{1}{2}$ months after onset of the infection. In the test at $1\frac{1}{2}$ -3 months the values were 920 cpm and 380 cpm respectively ($p < 0.05$). Responses measured later showed no significant differences between the 2 groups.

The range of responses to PPD was extremely large in both the arthritic and the nonarthritic groups. The

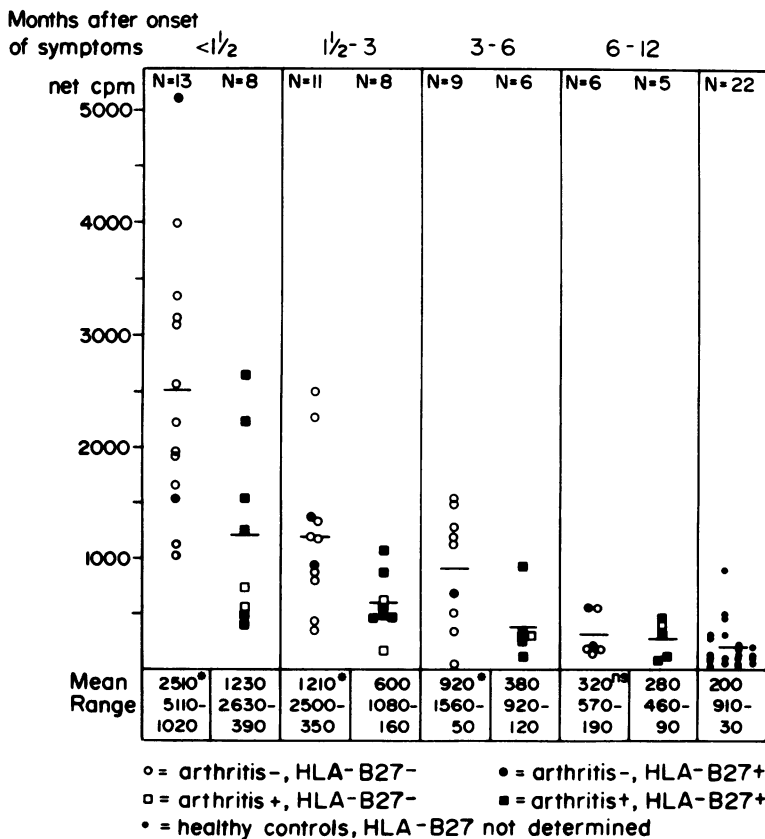


Fig. 1 *Lymphocyte transformation response to yersinia in patients with yersinia infection from the acute phase up to one year. N = number of patients. Statistically significant differences between the arthritic and nonarthritic patients: *p < 0.05, ns = Not significant.*

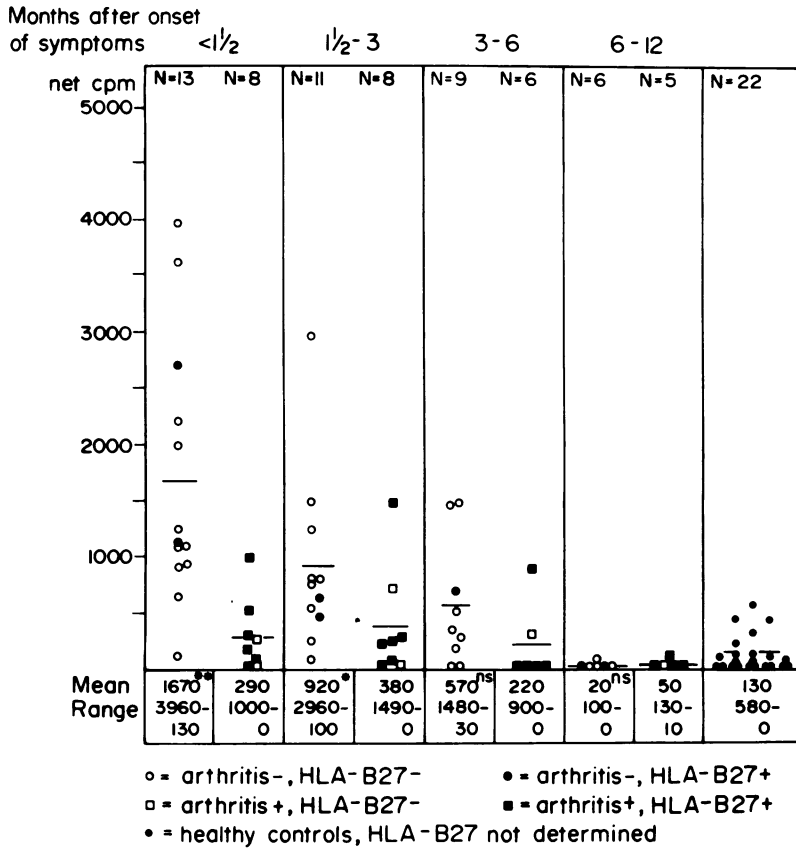


Fig. 2 Lymphocyte transformation response to *E. coli* in patients with yersinia infection from the acute phase up to one year. *N* = number of patients. Statistically significant differences between the arthritic and nonarthritic patients: **p*<0.05, ***p*<0.02, ns = not significant.

mean response of arthritic patients in the test at <math><1\frac{1}{2}</math> months was 3710 cpm (range 18 400–120), and that of nonarthritic patients 1500 cpm (range 4490–260). There were no significant differences in the responses to PPD in patients with or without arthritis, or in patients with or without HLA B27 during the whole period.

SK-SD stimulation induced a proliferation response in only a few patients. The mean response of the arthritic patients in the test at <math><1\frac{1}{2}</math> months was 770 cpm (range 2400–0), and that of nonarthritic patients 790 cpm (range 5030–0). No significant differences were observed in the lymphocyte responses to SK-SD in any groups.

LYMPHOCYTE TRANSFORMATION RESPONSES TO LECTINS

To compare the results of nonspecifically stimulated lymphocytes the lymphocyte responses were measured after mitogenic stimulation with PHA and con-A.

The responses to PHA and con-A were identical in all patient groups, whether arthritis developed or not

and whether HLA B27 was present or not. The mean responses to PHA in both patient groups ranged around 40 000 cpm and con-A responses ranged around 35 000 cpm.

CORRELATION OF THE HLA B27 ANTIGEN TO THE RESPONSES

To find a possible correlation between the presence of the HLA B27 antigen and the lymphocyte responses the patients were divided into HLA B27 positive and negative groups. No significant differences were observed in the lymphocyte responses to any of the bacterial antigens or the lectins tested.

Discussion

The main finding in the present study is that the patients developing reactive arthritis have weaker lymphocyte response to yersinia and *E. coli* than patients without this complication after yersinia infection.

Aho *et al.*¹³ have studied lymphocyte transformation responses of patients with yersinia infec-

tion using the same principles as we employed in this study. They could not find any differences between the arthritic and nonarthritic groups. The discrepancy may be explained by 2 facts. Lymphocyte transformation is a relatively insensitive test, and therefore the use of a suboptimal dose of antigen by Aho *et al.* may have resulted in poor or no responses. Secondly, it remained unclear how the tests were timed in relation to the onset of the infection, and a direct comparison between patients with and without arthritis cannot be carried out in the same way as in our material.

It is of special interest that our patients with arthritis showed significantly lower responses to both yersinia and *E. coli* when compared with patients without arthritis. Both of these bacteria possess an enterobacterial common antigen (ECA), as do salmonella and shigella,¹⁴ both of which can also produce reactive arthritis, especially in persons having the HLA B27 antigen.¹⁵⁻¹⁸ It is remarkable that the lymphocyte proliferation responses to yersinia and *E. coli* showed a similar decline in the responses with time. Furthermore recent studies¹⁹⁻²⁰ have suggested that klebsiella, also possessing the ECA,¹⁴ might be a causal agent in the development of ankylosing spondylitis. Taken together all these findings suggest a role for ECA in the pathogenesis of reactive arthritis and ankylosing spondylitis.

The normal and similar responses of lymphocytes to PPD, SK-SD, PHA, and con-A in both patient groups indicate that the weak responses in arthritic patients to yersinia and *E. coli* are a specific phenomenon. The present results do not indicate whether in arthritic patients specific suppressive factors are generated, or whether their lymphocytes respond more weakly to the intestinal yersinia infection. We have observed that gastrointestinal symptoms are milder in the patients developing arthritis than in others; in most instances these patients seek therapy because of the arthritis and the abdominal symptoms become apparent only in recollection. This may indicate that the inflammation due to yersinia in the bowel mucosa is weak in these patients, permitting prolonged persistence of the micro-organism. This would fit also with the observation²¹⁻²² that, in patients with yersinia arthritis, especially the serum IgA class antibodies against yersinia remain elevated for a long period.

Most studies of the relationship between cell-mediated immunity and the HLA B27 have been carried out on patients with ankylosing spondylitis. The results have been conflicting,²³⁻²⁶ probably owing to several factors. The aetiology of ankylosing spondylitis is unknown, and therefore the appropriate aetiological antigen is not available for the tests. But the responses of lymphocytes to this

aetiological antigen may well be specific. In the present work only the use of yersinia and *E. coli* revealed differences between the groups studied, all the other bacterial antigens and lectins proving to be nonspecific. Another important factor may be the time of testing. Ankylosing spondylitis is a chronic disease which starts insidiously, and thus in a late phase of the disease variations in the lymphocyte responses may be impossible to detect. In the present work the differences in the lymphocyte responses between the 2 patient groups disappeared 6 months after the infection. It is also worthwhile pointing out in this connection that in our material the weak lymphocyte responses were not primarily associated with the HLA B27 but rather with the developing of arthritis.

The results of studies of HLA B27 associated reactive arthritis may also elucidate the pathogenesis of ankylosing spondylitis. Sairanen *et al.*²⁹ showed that in patients with *Shigella flexneri*-induced Reiter's syndrome the most frequent changes 20 years later were ankylosing spondylitis (32%), chronic arthritis (18%), and iritis (7%), and only 20% of the cases were entirely asymptomatic. Reiter's syndrome was also strongly associated with HLA B27, which was found in 78% of the patients in their series.¹⁷ Our follow-up study of children with yersinia arthritis³⁰ revealed that some children had developed arthritis again after making a full recovery from the yersinia arthritis. Recurrent arthritis may not be actually associated with the earlier yersinia infection, but may illustrate the tendency of certain individuals, often with HLA B27, to develop arthritis.

Whatever the pathogenetic mechanisms of post-infectious reactive arthritis may be, several features in the immune system are altered in these patients. The present results indicate that the proliferative lymphocyte responses to the causative bacterium and *E. coli* are decreased, whereas earlier studies have shown that the opposite holds true for the specific antibody production. Granfors *et al.*²¹⁻²² measured IgM, IgG, and IgA class antibodies against yersinia in patients with yersiniosis. In the patients without arthritis the serum antibodies disappeared within 5 months, but in patients with arthritis the IgA class antibodies persisted up to 14 or 16 months. Furthermore, the peak values of anti-yersinia IgA were in direct correlation with the severity of arthritis. The contradictory findings in the humoral and cell-mediated immune responses indicate disturbances in the regulation of immune function in patients with arthritis. The aim of our future studies is to elucidate the mechanisms of such disturbances. Immune complex formation and function of phagocytic cells may be related to the persistence of the yersinia antigen. The role of the persisting antigen in immune

regulation—for example, effect on the T suppressor cell or T helper cell functions—and the relationship between these immunological findings and the disease susceptibility remain to be studied in patients with yersinia arthritis.

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