SHORT REPORT
LONGITUDINAL INVESTIGATION OF BACTERIUM-SPECIFIC SYNOVIAL LYMPHOCYTE PROLIFERATION IN REACTIVE ARTHRITIS AND LYME ARTHRITIS

C. FENDLER,*† P. WU,* U. EGGENS,† S. LAITKO,‡ H. SÖRENSEN,§ A. DISTLER,† J. BRAUN† and J. SIEPER*†

*Deutsches Rheumaforschungszentrum, †Department of Nephrology and Rheumatology, Klinikum Benjamin Franklin, ‡Rheumaklinik Buch and §Immanuel Hospital, Berlin, Germany

SUMMARY

Background. Antigen-specific lymphocyte proliferation of synovial fluid mononuclear cells (SF MNC) has been reported repeatedly in reactive arthritis and Lyme arthritis; however, less information is available on serial investigations of SF MNC in the same patients.

Methods. In this study, the synovial lymphocyte proliferation to *Yersinia*, *Chlamydia*, *Shigella* and *Borrelia burgdorferi* was investigated sequentially at different time points in 28 patients with reactive arthritis, undifferentiated oligoarthritis or Lyme arthritis responding to one of these bacteria.

Results. The same bacterium was always recognized in arthritis triggered by *Chlamydia*, *Shigella* or *Borrelia*, with much variation in the proliferative response. Only the *Yersinia*-specific responses changed specificity, suggesting that the proliferative response to *Yersinia* is non-specific in some patients.

Conclusions. Our data support the concept of a local antigen-specific T-cell response in reactive arthritis or Lyme arthritis but not the concept suggested by others that a switch to an autoimmune response takes place in long-standing disease.

Key words: Cellular immune response, Synovial fluid, Reactive arthritis, Lyme arthritis.

HLA-b27-associated reactive arthritis (ReA) occurs after an infection of the urogenital tract with *Chlamydia trachomatis* or of the gastroenteral tract with *Yersinia*, *Salmonella*, *Shigella* or other bacteria. Patients with undifferentiated oligoarthritis (UOA), predominantly involving the lower limbs in an asymmetrical pattern, often have a clinical pattern suggestive of ReA, but without a symptomatic clinical infection. In Lyme arthritis (LA), inflammation of the joint follows an infection with *Borrelia burgdorferi*. In all these arthritides, bacteria or bacterial antigens have been detected in the joint by various techniques, suggesting that these antigens drive the immune response locally [1–4]. This is supported by the finding of antigen-specific T-cell proliferation to all these bacteria in synovial fluid (SF) which is substantially lower in peripheral blood (PB) [5–9].

However, questions have been raised in the past concerning antigen-specific lymphocyte proliferation of SF T cells in ReA and LA patients. (i) Is this proliferation specific for the triggering bacterial antigen and is it specific for ReA or is it rather a reflection of the systemic immune response with a non-specific proliferation due to a higher level of activation of T cells and macrophages in the joint [10, 11]? (ii) When can a lymphocyte proliferation be called specific [8]? (iii) Does this proliferation remain specific over a long time during the arthritis or does it disappear in a chronic disease course [12]?

To address these questions, serial investigations of SF of ReA, LA or UOA patients were carried out at different time points in the same patients. In the great majority of the cases investigated, the lymphocyte proliferation remained highest to the same bacterium, although wide variation in the magnitude of proliferation was observed.

PATIENTS AND METHODS

Patients

Patients were recruited from four rheumatology clinics in the Berlin area. Paired samples of PB and SF were obtained from 216 patients with either ReA (*n*= 65), UOA (*n*= 133) or LA (*n*= 18). Puncture of a vein or joint was only performed when necessary for diagnostic or therapeutic reasons. Some of the patients have been reported on before in other studies [8, 13, 14].

Definitions

ReA was defined as oligoarthritis preceded by a clear history of urethritis or gastroenteritis in the previous 4 weeks. The triggering bacterium was identified either by a positive culture from stool or urethral swab, or by a significantly elevated antibody titre early in the arthritis [15]. UOA was defined as arthritis of unknown cause involving ≤ 4 joints including the knee, with no history of a preceding infection or extra-articular features suggestive of Reiter’s syndrome. Other rheumatic diseases were excluded by appropriate investigation. A diagnosis of LA was made if the

© 1998 British Society for Rheumatology
clinical picture was compatible and specific antibodies showed positive reactions as described before [16].

Cell separation and culture
Mononuclear cells (MNC) from PB and SF were separated as described previously [8], and triplicate wells were stimulated with some or all of the following agents: tissue culture medium alone (background proliferation), C. trachomatis serovar L1 (5 μg/ml), Yersinia enterocolitica O.3 and O.9 (3 μg/ml), Salmonella enteritidis (5 μg/ml), Shigella flexneri (5 μg/ml), Campylobacter jejuni (5 μg/ml), B. burgdorferi (5 μg/ml), tetanus toxoid (Behring, Marburg, Germany; 1 μg/ml) or pokeweed mitogen (Sigma, Poole; 1 μg/ml). All bacteria were grown as described before [8, 13] and heat inactivated at 60°C for 1 h. Stimulation was carried out with whole bacteria. Wells were pulsed with [3H]thymidine (7.4 kBq/well) for the last 18 h of culture and incorporation measured at day 6 as described previously [8].

Lymphocyte proliferation was expressed as [3H]thymidine incorporation in counts per minute. Results are shown as stimulation indices (SI), defined as the proliferation induced by an antigen divided by the background proliferation. The specificity of responses was defined as follows: in SF, SI > 5 were considered positive if the lymphocyte proliferation to the specific bacterial antigen was at least 2000 c.p.m. If responses to two or more antigens were positive, the highest SI had to be double the value of the next highest to be considered specific, otherwise this was regarded as a non-specific response. Subjects with SI < 5 for all pathogens were considered to be non-responders [8]. These stringent criteria had to be achieved at least once in each patient.

RESULTS
Altogether SF was investigated at least twice in 49 out of 216 patients. Of these patients, 28 showed a specific lymphocyte proliferation response to one of the bacteria tested at least once and were further analysed. In 14 patients, the stimulating bacterial antigen was Yersinia (diagnosis of ReA in patients 4, 11 and 12, and of UOA in patients 1, 2, 3, 5, 6, 7, 8, 9, 10, 13 and 14), in eight patients C. trachomatis (diagnosis of ReA in patients 15, 16, 18, 20, 21 and 22, and a diagnosis of UOA in patients 17 and 19), in three ReA patients Shigella, and in three LA patients B. burgdorferi (Fig. 1). In all patients with Chlamydia, Shigella or Borrelia as the stimulating bacterium, these antigens always gave the highest proliferation even if the criteria for specificity were not fulfilled, except in patient 21. This was an HLA-B27+ patient with two independent episodes of ReA: the first one after a preceding diarrhoea and the second one after a preceding urethritis caused by C. trachomatis. A specific lymphocyte proliferation to C. trachomatis was detected only during the second episode, but not during the first. Among the 14 patients with the highest lymphocyte proliferation induced by Yersinia, a higher proliferation to another bacterium could be detected at least once in four patients (patients 1, 3, 11 and 13).

Out of these 28 patients, the proliferative responses of five patients are shown in more detail in Fig. 2. Patient 2, who presented with the clinical picture of a UOA with repeatedly elevated IgA and IgG antibodies to Yersinia antigens, showed the highest response to Yersinia at seven different time points. However, only three times, at week 454, week 554 and week 595, were the specificity criteria met. In patients 15 and 16, the urethral/cervical smear was positive for C. trachomatis, both patients were antibody positive and in patient 15 Chlamydia could be detected in synovial fluid by polymerase chain reaction (PCR) [17]. The specificity criteria were met at two investigations in both of them, but not at a third. The two patients (26 and 28) with LA were antibody positive and B. burgdorferi was detected in their joint by PCR [17]. The specificity criteria were fulfilled only once in patient 26 (week 152) and twice in patient 28 (weeks 142 and 150).

DISCUSSION
The antigen-specific lymphocyte proliferation of T cells from SF to the triggering bacterium is a valuable research tool in ReA because it demonstrates the importance of the local T-cell response and allows the response to be characterized further, especially according to the T-cell cytokine pattern [16, 18, 19] and the identification of immunodominant antigens [20, 21]. Furthermore, it can be used for diagnostic purposes in patients with UOA [8] or juvenile chronic arthritis type II [13], although the sensitivity and specificity of the test are not yet clear. Doubts about the specificity of this test for the triggering bacterium have been expressed [6, 10, 11]. Through a series of experiments, we have shown in the past (i) that the antigen-specific T-cell frequency is higher in SF compared to PB [14]; (ii) that this T-cell reactivity is independent of whether the monocytes/macrophages come from PB or SF [14]; and (iii) that the higher proliferation in SF is not due to a higher proportion of CD45RO+ T cells compared to PB [22].

In this study, we now provide evidence that the synovial antigen-specific proliferation is always highest to the triggering bacterium in patients with Chlamydia-induced, Shigella-induced and Borrelia-induced arthritis at various time points. Especially for Chlamydia-induced and Borrelia-induced arthritis, this is also true several months and even years after the first manifestation of the arthritis. A constant local cellular immune response to the triggering bacterium is not unexpected for these bacteria, because of the long persistence of live Chlamydia and Borrelia in the joint, which has been repeatedly and convincingly demonstrated [1, 4, 23]. While two of the patients with Shigella-induced arthritis were investigated early in their disease, it is less clear why in patient no. 24 a Shigella-specific response could be detected up to 100 weeks, because this organism is less likely to live for a long time in vivo [24, 25].

In order to use the synovial lymphocyte proliferation for diagnosis, we have proposed specificity criteria in
the past [8]. In practice, these criteria were not fulfilled in some of the present patients (Figs 1 and 2). The level of the bacterium-specific proliferation seems to be related to various antigen-independent factors, such as the ratio of T cells to macrophages or the grade of T-cell activation [26]. It is also known that there is a substantial cross-reactivity of bacterial antigens, especially among the enterobacteria. However, because synovial lymphocyte proliferation to microbial antigens is also observed in patients without bacteria-related arthritis, specificity criteria for synovial lymphocyte proliferation would be essential if used as a diagnostic test, so resulting in unavoidable loss of sensitivity for this method. Therefore, our results argue against the use of antigen-specific lymphocyte proliferation for diagnostic purposes.

The detection of a higher level of proliferation to another bacterium in patients who have previously shown a specific response to Yersinia suggests that this organism can induce a false-positive synovial lymphocyte proliferation. By using Yersinia-specific recombinant proteins as stimulating antigens, we could indeed show recently that only about half of the ‘specific’ responses induced by whole Yersinia were specific [21]. In our experience, this seems not to be the case for Chlamydia or Borrelia.

There have as yet been only a few reports of serial pathogen-specific lymphocyte proliferation in the SF of patients with arthritis. Ford et al. reported on single patients with rheumatoid arthritis in whom the lymphocyte proliferation to mumps virus and adenovirus [27], rubella virus [28] or C. trachomatis [29] be related to various antigen-independent factors, such as the ratio of T cells to macrophages or the grade of T-cell activation [26]. It is also known that there is a substantial cross-reactivity of bacterial antigens, especially among the enterobacteria. However, because synovial lymphocyte proliferation to microbial antigens is also observed in patients without bacteria-related arthritis, specificity criteria for synovial lymphocyte proliferation would be essential if used as a diagnostic test, so resulting in unavoidable loss of sensitivity for this method. Therefore, our results argue against the use of antigen-specific lymphocyte proliferation for diagnostic purposes.

The detection of a higher level of proliferation to another bacterium in patients who have previously shown a specific response to Yersinia suggests that this organism can induce a false-positive synovial lymphocyte proliferation. By using Yersinia-specific recombinant proteins as stimulating antigens, we could indeed show recently that only about half of the ‘specific’ responses induced by whole Yersinia were specific [21]. In our experience, this seems not to be the case for Chlamydia or Borrelia.

There have as yet been only a few reports of serial pathogen-specific lymphocyte proliferation in the SF of patients with arthritis. Ford et al. reported on single patients with rheumatoid arthritis in whom the lymphocyte proliferation to mumps virus and adenovirus [27], rubella virus [28] or C. trachomatis [29] showed the highest proliferation to the same antigen over years. Although Chlamydia antigen was detected in synovial membrane in the patient who had the highest proliferation to Chlamydia, the role of these pathogens in the pathogenesis of RA is rather doubtful. There is only one article on a sequential follow-up of synovial lymphocyte proliferation in one patient with Yersinia-induced ReA [12]. This patient lost the initial antigen specificity during the chronic course of the arthritis after 2 yr. This has led the authors to speculate that an autoimmune response might have taken over. A switch from an antigen-specific to an autoimmune response has also been considered in long-standing LA [30].

In our study, several patients with Chlamydia-induced and Borrelia-induced arthritis could be observed for longer than 2 yr without losing specificity. Our data therefore do not support this concept, although recognition of both bacterial and cross-reacting self-antigens cannot be excluded. Furthermore, in proliferation assays, only a CD4+ T-cell response is counted and it is not excluded that cross-reacting antigens presented by the HLA-B27 molecule [31, 32, 33] might be recognized by CD8+ T cells.
In summary, in serial investigations of synovial fluid MNC, lymphocyte proliferation remains highest to the triggering bacterium; only *Yersinia* antigen can also stimulate a non-specific cellular response for unknown reasons.

**References**

10. Keat AC, Knight SC. Do synovial fluid cells indicate
Longitudinal investigation of bacterium-specific synovial lymphocyte proliferation in reactive arthritis and lyme arthritis