ANTIGEN-PRESENTING CELLS BUT NOT LYMPHOCYTES IN THE JOINT MAY INDICATE THE CAUSE OF REACTIVE ARTHRITIS

Antigen Presentation Research Group, Imperial College School of Medicine, Northwick Park Institute for Medical Research, Harrow HA1 3UJ, *Rheumatology Department, St Peter’s Hospital, Chertsey KT16 0PX and †Rheumatology Department, Northwick Park Hospital, Harrow HA1 3UJ

SUMMARY
T cells and antigen-presenting cells (APC) accumulate in the joint in reactive arthritis and there are reports that the T cells are a population selected for responsiveness to the causative agent. In this work, the latter view is questioned by detailed studies of the antigen specificities of the lymphocytes within the joint (SFMC) and peripheral blood (PBMC) of patients with reactive arthritis triggered by infection with Chlamydia trachomatis. Using a hanging-drop microculture system, SFMC displayed enhanced responses not only to antigens from the triggering organism, but also to other antigens, including PPD and tetanus toxoid, to which the patients were likely to have had prior exposure. No evidence was obtained for a dominant cross-reactive T-cell response to epitopes common to these antigen preparations, confirming the polyclonal nature of the infiltrate. In contrast to the broad specificity of the T-cell infiltrate, two experimental approaches indicated that APC within the joint carried chlamydial antigen. The failure of antigen-bearing APC to interact with T cells at this site may underlie the inability to clear microbial antigen from the joint.

KEY WORDS: Reactive arthritis, Antigen-presenting cells, Dendritic cells, T cells.

In some genetically susceptible individuals, infection of the genitourinary or gastrointestinal tracts, with a number of Gram-negative bacteria, appears to trigger an acute seronegative arthritis. The disease is believed to have an immunological rather than an infective basis since the joints are found to be sterile, and hence it has been termed reactive arthritis. The joint disease occurring following genital tract infection has been termed sexually acquired reactive arthritis (SARA) [6] and the major organism to be implicated has been Chlamydia trachomatis. Yersinia and Salmonella species are amongst those that have been associated with enteropathic reactive arthritis (ERA). The major genetically determined risk factor is the possession of the MHC class I allele HLA B27. The basis of the association with B27 has been the focus of much interest, but remains unresolved [7].

The development of disease in B27 transgenic rats with an acute seronegative arthritis. The disease is believed to have an immunological rather than an infective basis since the joints are found to be sterile, and hence it has been termed reactive arthritis. The joint disease occurring following genital tract infection has been termed sexually acquired reactive arthritis (SARA) [6] and the major organism to be implicated has been Chlamydia trachomatis. Yersinia and Salmonella species are amongst those that have been associated with enteropathic reactive arthritis (ERA). The major genetically determined risk factor is the possession of the MHC class I allele HLA B27. The basis of the association with B27 has been the focus of much interest, but remains unresolved [7].

Attempts to isolate viable organisms from the joints or detect nucleic acids have, with few exceptions, proved unsuccessful [10–12]. However, several groups have provided evidence that antigenic material derived from the triggering organisms may indeed localize to the joint [13–16]. This has strengthened the concept that a local immune response to bacterial components may be important in driving the inflammatory reaction. Several groups have reported, using bulk culture [2–4] or limiting dilution assays [17], that synovial fluid T cells from reactive arthritis patients display selectively enhanced reactivity to the same antigen. In contrast, Martin et al. [18] cited an enhanced peripheral blood T-cell response towards Chlamydia in patients with SARA versus those with uncomplicated urethritis as evidence for the involvement of this organism in their joint disease.

The significance of enhanced synovial fluid T-cell responses in the disease process is not clear. In this study, we have used a miniaturized hanging-drop culture system which enables responses to be examined in a multifactorial manner to examine in detail the antigen specificity of synovial fluid (SF) and peripheral blood (PB) T cells in patients with reactive arthritis and other arthropathies. We report that SF mononuclear cells (MC) often display enhanced reactivity to a panel of common recall antigens to which many patients are likely to be sensitized and that, compared with peripheral blood responses, this is not selective for the triggering antigen in reactive arthritis. Peripheral blood responses to all these antigens were also generally detectable, although different cell number requirements for PB and SF responses may mean that this is missed in experiments employing single response points.

In contrast to the non-specific nature of the T-cell infiltrate in SARA patients, we provide preliminary evidence in the second part of this study that SF antigen-presenting cells (APC) may be presenting chlamydial antigen in vivo. The ways in which these two observations may be reconciled are discussed.

MATERIALS AND METHODS

Patients
Fourteen patients were included in the study. Four...
had an undifferentiated seronegative arthritis (USA) with no evidence of either genitourinary or gastrointestinal infection. Seven patients fulfilled the criteria for SARA (i.e. a seronegative lower limb oligo- or monoarthritis with a urethritis or cervicitis preceding or contemporaneous with the arthritis). Four of the five SARA patients tested had high levels of serum antibodies to *Chlamydia trachomatis* (serovars D–K). Two patients had rheumatoid arthritis (RA) and one had Crohn’s disease. Further patient details are given in Table 1. Chlamydial serology was kindly performed by Dr J. Treharne, Institute of Ophthalmology, London, using a microimmunofluorescence method.

**Antigens**
*Chlamydia trachomatis*, serovars L1 and E, were grown in Buffalo Green Monkey Kidney cells and elementary bodies (EBs) partially purified on isopaque. EBs were irradiated (1000 Gy) prior to use. Purified protein derivative of *Mycobacterium tuberculosis* (PPD) was purchased from Statens Serum Institut (Denmark), tetanus toxoid (TT) in simple solution from Wellcome, and streptokinase–streptodornase (SKSD) from Lederle; *Candida albicans* particles were kindly supplied by Dr F. Manca, University of Genoa. Influenza virus X31 was egg grown and kindly supplied by Dr John Oxford, London Hospital. Recombinant antigens equivalent to 3/4, 1/2 and 1/4 of the chlamydial major outer membrane protein (MOMP) were kindly provided by Professor Michael Ward, University of Southampton [19].

**Cell isolation and culture**
Paired PB and SF samples were obtained from all patients. Heparin (10 U/ml) was used as anticoagulant for all SF samples and for most PB samples. The remaining PB samples were defibrinated. Similar results were obtained by both methods. Viscous SF were treated with 10 U/ml hyaluronidase. Preliminary experiments had established that hyaluronidase treatment did not affect SF responses.

Mononuclear cells were isolated by centrifugation on Ficoll-Paque (Pharmacia) and washed twice in HEPES-buffered RPMI-1640 containing 2% fetal calf serum (FCS). Viability was confirmed by trypan blue exclusion. Triplicate hanging-drop cultures were established in 20 μl of complete medium (Dutch modification of RPMI-1640 containing 10% fetal calf serum, 2 mM l-glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin) in inverted 60 well Terasaki plates (Falcon). Since it is never known what proportion of mononuclear cells may be responsive to different stimuli, a range of concentrates from 3000 to 100 000 cells/well was used. Antigens were added, as appropriate, in a volume of 1 μl and the plates inverted over saline in a plastic box in a humidified incubator maintained at 37°C with an atmosphere of 3% of CO₂. Tritiated thymidine ([³H]TdR; New England Nuclear—1 μl giving 1 mg/ml of thymidine at a specific activity of 2 Ci/mmol) was added 2 h before harvesting on day 5 using a hanging-drop blotting technique. Filters were washed with saline and 5% trichloroacetic acid, and dried with alcohol before being counted in a liquid scintillation counter. This technique, using a low-specific-activity thymidine in flooding conditions for a short pulse time, results in low counts but these reflect the level of DNA synthesis without the complication of limiting availability of thymidine or excessive radiation damage. In order to examine, simultaneously, several

### TABLE 1
Details of patients in Fig. 1

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Diagnosis</th>
<th>Genital tract symptoms</th>
<th>Chlamydia isolation</th>
<th>Chlamydia serology (IgG)</th>
<th>B27 status</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>SARA (4/52)</td>
<td>Cervicitis</td>
<td>+</td>
<td>1024 (1024)§</td>
<td>64 (64)    +</td>
</tr>
<tr>
<td>2</td>
<td>SARA (4/52)</td>
<td>NSU</td>
<td>+</td>
<td>64 (16)</td>
<td>16 (16)    +</td>
</tr>
<tr>
<td>3</td>
<td>SARA (5/52)</td>
<td>NSU</td>
<td>—</td>
<td>256 (256)</td>
<td>64 (64)    ND</td>
</tr>
<tr>
<td>4</td>
<td>SARA (6/52)</td>
<td>NSU</td>
<td>—</td>
<td>0 (0)</td>
<td>64 (32)    ND</td>
</tr>
<tr>
<td>5</td>
<td>SARA (2/52)</td>
<td>NSU</td>
<td>—</td>
<td>256 (256)</td>
<td>16 (0)     ND</td>
</tr>
<tr>
<td>6</td>
<td>USA (2/52)</td>
<td>—</td>
<td>—</td>
<td>0 (ND)</td>
<td>0 (ND)     ND</td>
</tr>
<tr>
<td>7</td>
<td>USA (2 yr)</td>
<td>—</td>
<td>—</td>
<td>0 (0)</td>
<td>64 (128)   +</td>
</tr>
<tr>
<td>8</td>
<td>USA (10/12)</td>
<td>—</td>
<td>—</td>
<td>32 (128)</td>
<td>0 (0)      ND</td>
</tr>
<tr>
<td>9</td>
<td>USA (6/52)</td>
<td>—</td>
<td>—</td>
<td>0 (0)</td>
<td>16 (32)    —</td>
</tr>
<tr>
<td>10</td>
<td>RA (16 yr)</td>
<td>—</td>
<td>—</td>
<td>0 (0)</td>
<td>0 (0)      ND</td>
</tr>
<tr>
<td>11</td>
<td>RA (15 yr)</td>
<td>ND†</td>
<td>ND</td>
<td>0 (0)</td>
<td>0 (0)      ND</td>
</tr>
<tr>
<td>12</td>
<td>Crohn’s (13 yr)</td>
<td>ND</td>
<td>ND</td>
<td>32 (18)</td>
<td>16 (16)    ND</td>
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<tr>
<td>13</td>
<td>SARA (2/52)</td>
<td>NSU</td>
<td>—</td>
<td>ND</td>
<td>ND         ND</td>
</tr>
<tr>
<td>14</td>
<td>SARA (5/12)</td>
<td>NSU</td>
<td>+</td>
<td>ND</td>
<td>ND         ND</td>
</tr>
</tbody>
</table>

SARA, sexually acquired reactive arthritis; seronegative lower limb oligo- or monoarthritis with urethritis or cervicitis; USA, undifferentiated seronegative arthritis; NSU, non-specific urethritis.

*History of NSU.
†ND, not done.
‡Gonococci isolated.
§Reciprocal of PB titre (reciprocal of SF titre). Serology was kindly performed by Dr J. Treharne, Institute of Ophthalmology, London.
variables (source of the cells, cell numbers and presence of antigen), an analysis of variance method employing the full cell concentration curve was employed to identify significant differences between responses. The use of this culture system and analysis of data have been described elsewhere [20].

Dendritic cell-rich low-density cells (LDC) were isolated as follows. Mononuclear cells at $5 \times 10^6$/ml were incubated overnight in 25 cm$^2$ tissue culture flasks (Falcon) in complete medium. Non-adherent cells were centrifuged over hypertoninc metrizamide (made as 14.5 g plus 100 ml complete medium) at 600 g for 10 min. LDC were recovered from the interface. These consisted of around 30% dendritic cells and the majority of the remaining cells were monocytes [1, 5]. Pellet cells were used as a source of lymphocytes (Ly).

**Derivation of T-cell blasts**

T-cell blasts were derived from PB and SF in a similar way. Mononuclear cells were cultured at $2 \times 10^6$/ml in 24 well tissue culture plates with chlamydial EBs (1:100) or PPD (10 μg/ml) for 5 days, and then split 1:4 and recultured in fresh medium containing 30 U/ml rIL-2. During the next 8 days, cells were split and re-fed with IL-2-containing medium as required. At this time, the blasts were no longer IL-2 responsive and were then washed extensively before use. Long-term T-cell lines were maintained by cycles of restimulation with antigen or mitogen [phytohaemagglutinin (PHA)] and irradiated (25Gy) PBMC (2 $\times 10^6$) and expansion with rIL-2.

**Experiments to determine whether antigen-presenting cells were presenting chlamydial antigen acquired in vivo**

Two approaches were used. First, the ability of SF LDC to stimulate autologous Chlamydia-specific and control T-cell lines was studied. LDC, enriched for dendritic cells (DC), and a highly potent source of APC were obtained as outlined earlier. High yields of LDC are generally obtained from SF [1, 5] and these were frozen in a minimal volume of medium containing 5% dimethyl sulphoxide (DMSO) by holding at -30°C in a glycerol bath for 30 min before transferring to liquid nitrogen. Too few PB LDC are obtained from most individuals for successful freezing. T-cell blasts enriched for PPD or chlamydial reactivity were cultured at $2 \times 10^4$/well with between $6 \times 10^4$ and $5 \times 10^4$ SF LDC in the absence of any added antigen. After 72 h culture in flat-bottomed microtitre plates, each well was pulsed with [³H]TdT for 4 h and harvested with an automated harvester. [³H]TdT incorporation was assessed by standard liquid scintillation counting.

Secondly, the antigen specificity of T cells clustering with SF LDC was analysed. PB Ly (2 $\times 10^6$/well) and SF LDC (2.25 $\times 10^6$/well) were cultured together in 24 well tissue culture plates for 48 h. After this time, clustered and non-clustered lymphocyte fractions were separated by 1 g sedimentation through a 30% serum gradient [21]. After washing, responses of both fractions to EBs and PPD were assayed in hanging drops, as previously described. No additional APC were added at this stage.

**RESULTS**

**Cultures of mononuclear cells (MC) display different cell number requirements for PB and SF cells**

PBMC and SFMC from all 12 patients displayed significantly different cell number requirements in response to PPD, *Chlamydia* or tetanus toxoid when compared across a cell concentration range from 3000 to 100 000 cells/well (Fig. 1a–c, significantly different responses marked with an asterisk). In particular, with cells from eight patients (nos. 1, 2, 4, 5, 7, 9, 10 and 12), fewer SFMC were required to reach optimal or supraoptimal responses and this can be interpreted as an enhanced response compared with PBMC. In addition, the interaction of cell number with antigen concentration was different for PBMC and SFMC (data not shown). These data caution very strongly against comparing PBMC and SFMC on the basis of single points, and these will often fall on a different part of the respective response profiles. Previous work from this laboratory has shown that the bell-shaped curves obtained in these experiments are not confined to the hanging-drop culture system and are not due to limiting cell culture conditions [20, 22, 23].

In SARA patients, SFMC respond to a range of recall antigens as well as *Chlamydia trachomatis*

The pattern of enhanced response of SFMC as evident from the requirement for fewer cells to reach peak response levels was not confined to a particular antigen, but was observed with a panel of bacterial antigens: *Chlamydia*, PPD and tetanus toxoid (Fig. 1a–c). This was true for the SARA patients, in two of whom *Chlamydia* had been unequivocally implicated as a trigger of arthritis since *C. trachomatis* infection was proven by culture of the organism.

Both PBMC and SFMC responses were detected to PPD in all but one patient (Fig. 1), but responses to tetanus toxoid and *Chlamydia* were more variable. Reactivity to *Chlamydia* was most marked amongst the SARA and USA patients; good responses to this antigen may thus be a feature of both PB and SF lymphocytes, and reflect a recent exposure to this antigen. However, weak responses were detected in the RA patients, and both PBMC and SFMC from the Crohn's disease patient responded very strongly.

In an attempt to eliminate the possibility that the wide-ranging reactivity of SFMC from SARA patients was due to reactivity to one or more dominant epitopes shared between the various antigen preparations, a polyclonal T-cell line was selected from the SFMC of patient 1 with *C. trachomatis* antigen and tested for cross-reactivity with other antigens which stimulated the unselected SFMC population. The T-cell line (100% CD3+, 50% CD4+, 45% CD8+) displayed strong reactivity to *C. trachomatis* 78a (serovar E) and cross-reacted with serovar L1 elementary bodies, but
FIG. 1.—(a–c) Proliferative responses of PBMC (●) and SFMC (○) from patients with inflammatory arthritis in the presence of no antigen, C. trachomatis L1 EB (1:1000), PPD (10 μg/ml) or TT (1:10⁵). Proliferation in the presence of antigen is significantly (P < 0.05) greater than in control cultures unless marked ns. In panels marked with an asterisk, the response curves for PBMC and SFMC differed significantly from each other (P < 0.05).
did not recognize PPD or influenza virus which were stimulatory for the unselected SFMC population (Figs 1a and 2). Some of the T cells in this line were reactive to the chlamydial MOMP. A recombinant MOMP antigen, equivalent to 3/4 of the native molecule, was stimulatory but two smaller fragments which lack the second variable segment (VSII) (1/2 and 1/4 length) were not positive, indicating the presence of a T-cell epitope in the region unique to the larger fragment or alternatively different processing, by antigen-presenting cells, of the antigens of different size. In addition, none of a panel of nine Chlamydia-reactive T-cell clones grown from the SF of an RA patient reacted with PPD or TT (in preparation), and polyclonal lines from the PB of a healthy donor raised against either Chlamydia or PPD recognized only the antigen they were grown with and did not cross-react with the other (not shown).

**Fig. 2.**—Antigen reactivity of a polyclonal T-cell line derived from the synovial fluid of patient no. 1 (SARA).

**Fig. 3.**—Evidence that SF LDC from SARA patients are presenting chlamydial antigen acquired *in vivo*. In Experiment 1, T-cell blasts were derived from SF (a) or PB (b) of a SARA patient (patient 13). (c) Reactivity of T-cell blasts from the PB of a second SARA patient (patient 1) and (d) reactivity of PB blasts from a control, as follows: (●) SF LDC + CT blasts; (○) SF LDC + PPD blasts; (■) PB LDC + CT blasts; (△) PB LDC + PPD blasts.
Are LDC from the joints of SARA patients presenting chlamydial antigen?

We first sought evidence that SF LDC from SARA patients are presenting chlamydial antigen acquired \textit{in vivo} by raising autologous T-cell blasts against either chlamydial or PPD antigens and testing the ability of SF LDC to stimulate them in the absence of added antigen (Fig. 3). In the first experiment (Fig. 3a and b), blasts were raised from both SF (Fig. 3a) and PB (Fig. 3b) of a SARA patient. In both cases, SF LDC stimulated the blasts raised against \textit{Chlamydia}, but not those raised against PPD. There were insufficient PB LDC to freeze for testing. This was possible with a second SARA patient (Fig. 3c): SF LDC, but not PB LDC, stimulated the \textit{Chlamydia} blasts. They did not stimulate the PPD blasts. When this type of experiment was performed using PB cells from a healthy donor (Fig. 3d), there was no differential in the ability of the LDC to stimulate either blast population, although there was some stimulation of both if sufficient LDC were added. In the second type of experiment, PB Ly were allowed to interact with SF LDC for 48 h, and then the clustered and non-clustered fractions obtained. SF Ly were not used because of the block in the interaction of these cells with DC [5]. The non-clustered fraction contained T cells capable of recognizing both chlamydial EBs and PPD (Fig. 4) with a similar response to both antigens. In contrast, in the clustered fraction, a response to PPD was undetectable and there was a 20-fold increase in the response to \textit{Chlamydia} (Fig. 4). These findings indicated that \textit{Chlamydia}-reactive T cells have been selected from the starting population by the presence of chlamydial antigen on the joint DC. These data are preliminary, but consistent with the notion that APC in the joints of SARA patients have acquired chlamydial antigen \textit{in vivo} and that this is retained during the purification of the cells in a form that can be detected by T cells \textit{in vitro}.

**DISCUSSION**

There were two major findings in this study. First, mononuclear cells from the SF displayed enhanced reactivity to recall antigens, but this enhancement was not antigen-specific even in those well-characterized patients with SARA in whom chlamydial infection was regarded as a trigger of their joint disease. The second and somewhat paradoxical observation was the preliminary evidence for the presence of chlamydial antigen on the surface of APC from the joints of SARA patients in a form recognizable by T cells.

Studies from several other groups have noted enhanced responses of SFMC to the triggering organisms in reactive arthritis [2–4, 17]. It is not yet clear whether this enhanced response is due to a lower activation threshold for synovial T cells, to enhanced APC function in this population, to an increase in the frequency of T cells reactive to the antigen in question or to a combination of these possibilities. There is also evidence that PBMC responses may be selectively depressed in reactive arthritis [24]. T cells isolated from the joint are almost exclusively of memory phenotype [25], in contrast to PB where approximately half of the T cells appear to be phenotypically naive [26]. Naive and memory T cells are reported to have different activation requirements [27], and hence enhanced responses in SFMC may simply be due to an increase in the proportion of readily activated memory cells. However, our previous studies have indicated a defective interaction of SF T cells with DC [5] and responses of PB T cells are not enhanced by depletion of naive cells [28].

That the enhanced response of SFMC is due, at least in part, to the APC at this site is suggested by a number of observations. SF is a very rich source of APC and, in particular, DC [29–31]. Indeed, SF are enriched for DC compared with paired PB and these DC are as potent, or more potent, on a per cell basis than their counterparts in PB [1]. Furthermore, Life et al. [32] reported that a crude preparation of SF APC was able to unmask peripheral blood T-cell responses to bacterial antigens which were not detectable when PB APC or unfractionated PBMC were used. In support of the concept that APC are responsible for the differences in T-cell responsiveness, our preliminary data indicated that purified T cells from PB and SF no longer show differences in response dynamics when reconstituted with fixed numbers of APC (not shown).

Limiting dilution analysis suggested a 2-fold increase in the frequency of the T cells' response to the triggering organism in SF compared with PB [17]. However, in these studies, the responding T cells were not rigorously depleted of APC and so a contribution of these cells cannot be excluded. Earlier work, employing bulk culture assay, suggested that responses to the triggering organisms were largely confined to SF [2–4, 33]. However, these conclusions were based on
analysis of single response points and the current study shows that this can be a misleading way of comparing PB and SF responses: it is evident from Fig. 1 that, for many patients, depending on where in the cell number range a single point is selected, the answer that SFMC responses were greater than, equivalent to, or less than PBMC responses could be obtained. Differences in the dynamics of lymphocyte proliferation have also been observed between cells from the peripheral blood of healthy controls and RA patients in response to mitogenic lectins [34], in multiple sclerosis patients during the course of disease [35], and in cells from patients with Behçet's disease before and after immunosuppressive treatment [36]. In all these studies, it was again shown that comparisons on the basis of single response points could be misleading and could indeed yield contradictory results depending on the conditions employed.

Finally, heightened responses may not be a property unique to MC from the joint or to patients with arthritis, but may be true of cells from other sites of inflammation [37].

Whatever the mechanism of the enhanced SFMC responsiveness, the current study indicates that it is not antigen specific since responses to common recall antigens PPD and TT were increased. The difference between this and earlier studies where SF responses appeared to be more restricted probably lies in the choice of control antigens. We have used antigens to which most individuals are likely to be sensitized, whereas others have studied patients with arthritis following genital (SARA) or gastrointestinal (ERA) infection and compared to MC reactivity to organisms associated with one or other infection site. It is possible that the finding of SFMC reactivity to organisms which infect the genital tract in the SARA patient group, but not those with ERA, could simply reflect the antigenic history of these individuals rather than being related directly to the disease process. It is notable that, in the study of Ford et al. [38], synovial fluid mononuclear cells from both reactive and rheumatoid arthritis patients responded very strongly to a mumps virus antigen preparation—an antigen to which most individuals in their study population were sensitized—as well as to the suspected triggering antigen.

The finding that at the time clinical disease is apparent the SF T-cell infiltrate is polyclonal is consistent with the failure to find marked oligoclonality in T-cell receptor usage in unselected SF T cells [39–42], with the low frequency of T cells responding to the triggering antigen in reactive arthritis [17] and with the observation that synovial lymphocytes produce antiviral antibody following immunization of RA patients with influenza virus [43]. It remains possible that recruited cells are masking a small number of T cells specifically responding to antigen localized in the joint or that the infiltrate is more specific earlier in the disease, perhaps before clinical changes are clinically apparent.

Given the polyclonal nature of the infiltrate, the significance of finding T cells reactive to MOMP in SF is not known. It is notable, however, that in a more extensive study of 10 SF, T-cell clones from a SARA patient by Hassell et al. [44], reactivity to antigens of 18 and 300 kDa, but not MOMP, was identified.

Reactivity to C. trachomatis was not confined to the SARA patients, although it was more common in this group. It is conceivable that this reactivity is due to sensitization with a cross-reactive organism such as Chlamydia pneumoniae. Infection with C. pneumoniae is common [44, 45] and may itself trigger reactive arthritis [46]. Interestingly, PBMC and SFMC from the patient with Crohn's disease responded very strongly to chlamydial antigen since a link between C. trachomatis infection and inflammatory bowel disease has occasionally been proposed [47].

The second finding in the current study was evidence for the presence of chlamydial antigen in or on APC isolated from the joints of SARA patients. Activation of T cells by DCs is a two-stage process. Initially, DC cluster T cells in an antigen-independent manner, but with time these clusters become enriched for T cells reactive to whatever antigen is being presented by DC [21, 48]. We found that clusters formed between SF DC and PB T cells from a patient with SARA were markedly enriched for chlamydial reactivity. SF DC were also able to stimulate autologous T-cell lines raised against Chlamydia, but not PPD, in the absence of added antigen.

These data support the suggestion that DC are able to retain antigen in an immunogenic form for long periods of time. There is considerable evidence from experimental systems that, following immunization of animals, DC can be isolated from lymphoid tissue bearing the antigen in a form that can be seen by T cells [48–52]. For instance, in the study of Holt et al. [51], DC were isolated from the lungs of rats immunized, by aerosol exposure, with ovalbumin. These APC, but not those from non-immune animals, were capable of stimulating ovalbumin-reactive T-cell lines in vitro in the absence of any exogenous antigen. The current findings are preliminary. In particular, the specificity of the phenomenon for patients with SARA needs to be established. Attempts to do this so far have largely failed due to an inability to grow T-cell blasts to Chlamydia from other patients (not shown). Another problem lies in the low numbers of LDC obtained and the resulting problems of storing these cells for use in restimulation experiments once cell lines are established. However, if the current findings are confirmed, the approach could be extended to using T-cell lines and clones with more defined specificity in order to identify the in vivo acquired antigen more precisely. The approach should prove useful in identifying target antigens in other autoaggressive diseases.

The data add to the controversy questioning the role of T cells in the development of inflammatory arthritis [53, 54]. They show that APC presenting specific antigen may accumulate in the joints and that T cells responsive not only to that antigen, but also to other antigens, accumulate at this site. However, earlier work showed a lack of interaction between APC and T cells
taken from the joints [5]. Freshly isolated SF APC frequently caused marked stimulation of PBLy, but failed to stimulate SFLy. The current observations make this lack of interaction surprising. The failure of interaction may underlie the inability to clear antigen at that site. A local block in interaction between APC and lymphocytes may thus be an important feature of the cells accumulating within the joint fluid in inflammatory arthritis.

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