

T cells are responsible for the enhanced synovial cellular immune response to triggering antigen in reactive arthritis

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SUMMARY

In reactive arthritis (ReA) there is specific proliferation of synovial fluid (SF) mononuclear cells (MNC) to the triggering bacterial antigen; comparatively little or no response is seen in peripheral blood (PB). To investigate the mechanism of this elevated local immune response, we examined patients with typical ReA who showed an enhanced antigen-specific synovial immune response in bulk culture. Using separated fractions of T cells and antigen-presenting cells (APC) from PB and SF we showed that the synovial T cells rather than SF APC are responsible for the specific proliferation. By limiting dilution analysis, the frequency of T cells responding to the specific antigen was found to be significantly increased compared with the frequency of irrelevant antigen-specific T cells. Furthermore, the frequency of T cells responding to the specific antigen was higher in SF (between 1/619 and 1/4846, mean 1/2389) than in PB (between 1/1286 and 1/16279, mean 1/7350). We conclude that the specific synovial cellular immune response in ReA is mainly due to an expansion of antigen-specific T cells within the joint. However, the non-specific hyper-reactivity of SF T cells and differences between SF and PB APC may make a more minor contribution.

Keywords T cell frequency synovial fluid reactive arthritis

INTRODUCTION

Reactive arthritis (ReA) is defined as an aseptic inflammatory arthritis triggered by genitourinary infection with *Chlamydia trachomatis* (CT) or by enteric bacteria like *Yersinia*, *Shigella*, *Salmonella* and *Campylobacter* [1]. Several groups, including ourselves, have demonstrated a specific cellular immune response to the triggering bacterium in synovial fluid (SF) mononuclear cells (MNC) from patients with CT [2,3], *Yersinia* and *Salmonella* [4,5] induced ReA. Little response was seen with paired peripheral blood (PB) MNC. This bacteria-specific synovial response is likely to be triggered *in vivo* by the bacterial antigen which has been demonstrated in the joints of patients with arthritis due to *Yersinia*, [6,7], *Salmonella* [8] and CT [9,10].

The mechanism for the specific response of SF MNC to bacterial antigens in ReA, and their enhanced responsiveness compared with paired PB MNC, is not clear. Whilst the simplest hypothesis is that, in the ReA joint, there is an increased number of SF T cells which are specific for the triggering bacteria, differences between SF and PB T cells or antigen-presenting cells (APC) could also play a role. SF APC in inflammatory arthritis differ from PB APC in their differentiation, HLA-DR and

adhesion molecule expression and also in the relative proportions of the various APC subtypes [11,12]. Furthermore ReA PB T cells show an enhanced response to bacterial antigens if cultured with SF rather than PB APC [13].

There are also major phenotypic and functional differences between SF and PB T cells. SF T cells are primarily of the CD45RO⁺ subset [14], thought to contain most of the T cell memory [15,16], and many are also activated as judged by other markers [14,17]. In contrast, only half PB T cells are CD45RO⁺ and few bear other activation markers. T cell memory has two components, increased number of antigen-specific T cells and hyper-reactivity of the memory T cells to antigen [18]. Thus, memory T cells of diverse antigen specificities respond better to any antigen than naive cells with the same range of specificities. It has been proposed [19,20] that the enhanced ReA SF MNC may be due solely to the predominance of memory T cells in SF and that its specificity for triggering antigen arises because T cells are recruited into the joint according to their prevalence in the systemic circulation, which is highest for T cells expanded by a recent or ongoing infection.

To distinguish among the possibilities discussed above we performed two types of experiments. Using separated populations of PB and SF APC and T cells, we demonstrated that synovial T cells and not synovial APC are responsible for the enhanced antigen-specific MNC proliferation in ReA SF.

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Table 1. Characteristics of patients

Patient no.	UReA	EReA	Antibodies	Culture*	B27	LP†
1	-	+	<i>Yersinia enterocolitica</i>	-	+	11.3/ <i>Yersinia enterocolitica</i>
2	+	-	CT	-	+	7.7/CT
3	-	+	<i>Yersinia enterocolitica</i>	-	-	15.5/ <i>Yersinia enterocolitica</i>
4	+	-	CT	CT	+	27/CT
5	-	+	-	-	+	20/Shigella
6	-	+	-	-	-	27/Shigella
7	-	+	-	-	+	28/Shigella
8	+	-	-	-	-	-
9	-	+	-	-	+	-
10	-	+	CT	-	-	-
11	+	-	-	-	+	-

UReA, Urogenic reactive arthritis; CT, *Chlamydia trachomatis*; EReA, enteral reactive arthritis.

* From urethral/cervical smear and stool.

† Synovial lymphocyte proliferation to the specific antigen, expressed as stimulation index.

Furthermore, we confirmed that expansion of specific T cells within the joint rather than T cell hyper-reactivity was the major factor since, by limiting dilution analysis, T cell frequency responding to the specific antigen was increased in SF compared with PB in four out of five patients.

PATIENTS AND METHODS

Patients

Paired samples of SF and PB were obtained from 11 patients (2 female and 9 male; mean age 31.7 ± 13.5 years) with ReA. ReA was defined as oligo- or monarthritides following a clear cut symptomatic infection, either urethritis or gastroenteritis, in the preceding 5 weeks [1]. Seven patients had enteric ReA (EReA) with a preceding gastrointestinal infection and four patients urogenic ReA (UReA) with a preceding urogenital infection. The patients' characteristics are shown in Table 1. Antibodies were determined by immunoperoxidase assay for CT [21] and immunoblotting for *Yersinia* [22].

Definition of responder and non-responder patients

In preliminary experiments, the proliferation, measured as described below, of each patient's SF MNC was tested to the following ReA-related antigens: CT, *Yersinia*, *Shigella*, *Salmonella*, *Campylobacter* and *Borrelia burgdorferii*. The response was expressed as a stimulation index (SI), defined as proliferation induced by antigen divided by proliferation to medium alone. $SI \geq 5$ to an antigen in the absence of response to any other was judged a specific response (Table 1). A patient with $SI < 5$ and $ct/min < 2000$ for all pathogens was considered a non-responder.

Seven patients (five EReA patients: 1, 3, 5, 6, 7; two UReA patients: 2 and 4; disease duration 3.7 ± 1.9 weeks) had a specific cellular immune response to a single ReA-associated bacterial antigen and four (three EReA patients: 8-10; one UReA patient: 11; disease duration 1.3 ± 1 year) had no response to any antigen.

Cell separation

MNC were separated as previously described [2] from paired samples of PB and SF by density gradient centrifugation

(Lymphoprep, Nycomedas, Norway) and resuspended in tissue culture medium (TCM) comprising RPMI-1640 (GIBCO, Paisley, UK) with a 10% fetal calf serum (FCS, GIBCO), penicillin/streptomycin (100 U/100 µg per ml; Biochrom KG, Berlin, Germany) and glutamine (2 mM/ml; Biochrom).

Separation of T and non-T cells for cell-mixing experiments was performed using adherence or rosetting with sheep erythrocytes. For separation of adherent and non-adherent cells, PB or SF MNC were incubated in TCM for 90 min at 37°C on a plastic Petri dish (Nunc, Roskilde, Denmark). Non-adherent cells (>80% CD3⁺ by immunofluorescence) were removed by aspiration and subsequent washing and were used as responder T cells in the assays. Adherent cells (<5% CD3⁺ by immunofluorescence) were then scraped off the Petri dish with a disposable cell scraper (Costar, Cambridge, MA); these were irradiated at 40 Gy and used as APC. Rosetting with erythrocytes was performed as previously described [23]. The T cell population was >90% CD3⁺ by immunofluorescence. Non-T cells (<5% CD3⁺ by immunofluorescence) were irradiated at 40 Gy and used as APC. Before use, all cell populations were resuspended in TCM.

Cell culture and proliferation assays

Cells were aliquoted into 96-well plates. Unseparated MNC were cultured at 100 000 cells/well. In experiments with separated populations, 5000 irradiated PB or SF APC (adherent cells or rosette-negative cells) were cultured with 50 000 PB or SF responder T cells (non-adherent or rosette-positive cells).

Wells were stimulated, in triplicate, with the following agents:

Tissue culture medium alone (background proliferation)

CT serovar L1 (5 µg/ml), grown and purified as described [24]
Yersinia 0:3 and 0:9 (3 µg/ml), grown in trypticase soya bouillon over 48 h and washed in PBS

Salmonella enteritidis (5 µg/ml), *Shigella flexneri* (5 µg/ml), *Campylobacter jejuni* (5 µg/ml), grown in broth, boiled for 1 h to inactivate them, and washed in PBS

Borrelia burgdorferi (5 µg/ml), isolation B29 from a Berlin tick, grown in Kelly's medium and washed in PBS [25]

Tetanus toxoid (TT, Behring, Marburg, Germany) 1 µg/ml
Pokeweed mitogen (PWM, Sigma, Poole, UK) 1 µg/ml.

Cells were cultured for 6 days at 37°C in a 5% CO₂ incubator and ³H-thymidine (Amersham, UK; 0.2 µCi per well) incorporation measured as previously described [2]. Results are expressed as SI or as incremental ct/min (proliferation to a stimulus – that to medium alone). Optimal doses and times for assays were investigated in preliminary experiments (data not shown). Results are expressed as mean ± 1 s.d.

Limiting dilution assay (LDA)

Method. LDA were performed using cell populations separated by adherence. As responder cells, SF or PB non-adherent cells were used at six different concentration levels in each experiment as shown below. As APC, irradiated adherent cells were used at 10 000 cells/well; to ensure equivalent antigen-presenting capacity, these cells were always obtained from PB. The concentrations of non-adherent cells used were between 625 and 20 000 per well. Seventy-two replicate wells were set up at each responder cell dilution, 24 stimulated with the relevant specific antigen, 24 with an irrelevant ReA-associated antigen and 24 without antigen (background wells). As a positive control, three further wells containing 10 000 non-adherent cells and 10 000 irradiated adherent cells were stimulated with PWM.

Statistical analysis

A culture well was scored as positive when the ct/min measured for that well exceeded the mean ± 3 s.d. of the 24 corresponding wells without antigen. Results were calculated using maximum likelihood analysis based on the Poisson distribution [26]. χ^2 and confidence intervals (CI) were calculated using a computer program based on GLIM 3.12 (Royal Statistical Society, London). A cell concentration resulting in 37% negative wells corresponds to the frequency of responder cells in the population being analysed. Frequencies of responder cells in two populations are considered as different at the 5% significance level if the 95% CI of the calculated values do not overlap. *P* values for single-hit kinetics are calculated from χ^2 tables allowing *n* – 1 degrees of freedom where *n* is the number of informative cell concentration levels, that is, the number, out of the six set up, which gave different proportions of positive wells. *P* values > 0.05 are consistent with single-hit kinetics [26].

RESULTS

Proliferative responses of unseparated synovial and peripheral blood MNC: choice of specific and irrelevant stimulatory antigens

Choice of specific and irrelevant antigens. In preliminary experiments (data not shown), proliferation of PB and SF MNC from the 11 ReA patients had been tested to the ReA-related organisms CT, *Yersinia*, *B. burgdorferi*, *Salmonella*, *Shigella* and *Campylobacter*. Seven patients had been selected as specific responders to a single organism. Five of these had EReA comprising patients 1 and 3, who responded specifically to *Yersinia*, and patients 5, 6 and 7, who responded specifically to *Shigella*. Two of the seven, patients 2 and 4, had UReA and responded specifically to CT. In these seven patients, all subsequent experiments were performed testing proliferation to the specific organism and an irrelevant ReA-associated bacterium to which the patient had shown no response. Patients 1 and 3 were tested with *Yersinia* (specific) and CT (irrelevant),

patients 2 and 4 with CT (specific) and *Yersinia* (irrelevant) and patients 5, 6 and 7 with *Shigella* (specific) and CT (irrelevant). Four patients, three with EReA and one with UReA, were chosen because they did not respond to any tested organism and, in this investigation, were studied with two antigens to which they had shown no response.

Proliferative responses of synovial and peripheral blood MNC. Table 2 (patients 1, 2, 4, 6, 7) and Fig. 1 (patients 1, 3, 4, 5, 6, 7) show the proliferation of unseparated PB and SF MNC to the specific and irrelevant bacterial antigens in the specific responder group. Only the data from the specific responders are shown. As can be seen, the specificities established in preliminary work were maintained in these experiments. In all responder patients the bulk proliferation to the triggering bacterium in SF was significantly increased compared to PB. Retesting of the non-responder patients confirmed their lack of response to the chosen organisms (data not shown).

Synovial T cells proliferate better than PB T cells to the triggering bacterium whether co-cultured with PB or synovial APC

Separation of T cells and APC by plastic adherence. To assess whether the increased response to the triggering organism by ReA SF MNC compared to PB MNC was due to the T cell or the APC subset, SF and PB MNC were separated into T cell and APC populations and both SF and PB T cells were co-cultured with both SF and PB APC. In initial experiments (Fig. 1a), the separation was carried out by adherence to plastic, adherent cells being used as APC and non-adherent cells as T lymphocytes. As can be clearly seen (Fig. 1a), the SF T cells of responder patients showed proliferation to the specific antigen, comparable to that of the unseparated SF MNC, whatever the source of APC used. In contrast, the proliferation of PB T cells remained low whether SF or PB APC were used. No proliferation was seen to the irrelevant antigen with any combination of APC and T cells in the responder patients and, similarly, no proliferation was seen to any tested antigen in the non-responder group (data not shown).

Separation of T cells and APC by rosetting with sheep erythrocytes. To ensure that these results were not related to the technique of separation, in three patients parallel experiments were performed where the T cells and APC were separated by rosetting with sheep erythrocytes (Fig. 1b). Again the SF T cells demonstrated a strong triggering antigen-specific proliferation with either PB or SF non-T cells as APC, whereas, whatever the source of APC, the proliferation of PB T cells was low. The level of proliferation of the SF T cells was surprisingly similar whether separation was carried out by adherence or by rosetting and paralleled that of the unseparated SF MNC.

Increased frequency of triggering antigen-specific T cells in the SF compared with the PB in ReA

Limiting dilution assay techniques. From the above experiments, it was clear that the enhanced triggering antigen-specific T cell response in ReA SF was primarily related to the SF T cells and not to the APC population. To assess directly whether there was an accumulation of antigen-specific T cells within the joint, the frequency of antigen-specific T cells in PB and SF was compared by LDA. T and APC populations were separated by adherence and SF or PB T cells were plated out, under limiting dilution conditions, together with a fixed number of PB APC. In

Table 2. Antigen-specific T cell frequency in synovial fluid (SF) and peripheral blood (PB) from reactive arthritis patients showing a triggering antigen-specific response in bulk culture

Patient	Antigen	Proliferation* MNC (mean \pm 1 s.d.)	Reciprocal T cell frequency (95% CI)	No. lev	χ^2	P
1	<i>Yersinia enterocolitica</i>	PB 2505 \pm 2000	1286 (962–1719)	4	4.4	>0.05
		SF 22 158 \pm 1482	619 (459–833)	4	6.8	>0.05
	CT	PB 0	> 215 000 —	—	—	—
		SF 10 224 \pm 1271	148 888 (62 161–356 612)	3	2.8	>0.05
2	CT	PB 15 820 \pm 272	5000 (3836–6386)	5	8.1	>0.05
		SF 25 764 \pm 310	4846 (3781–6212)	6	9.6	>0.05
	<i>Yersinia enterocolitica</i>	PB 4895 \pm 1000	9653 (7192–12957)	5	6.1	>0.05
		SF 7231 \pm 350	7233 (5536–9449)	6	6.0	>0.05
4	CT	PB 6177 \pm 2500	2368 (1817–3086)	5	5.3	>0.05
		SF 18 984 \pm 1645	1184 (884–1587)	4	7.5	>0.05
	<i>Yersinia enterocolitica</i>	PB 3093 \pm 228	3990 (3125–5093)	6	10.7	>0.05
		SF 5932 \pm 1108	5708 (4247–7673)	5	1.3	>0.05
6	Shigella	PB 0	16 279 (11 319–23 413)	4	3.9	>0.05
		SF 25 559 \pm 7000	3370 (2577–4408)	5	6.4	>0.05
	CT	PB 0	38 501 (22 853–64 863)	4	2.6	>0.05
		SF 319 \pm 185	38 501 (22 853–64 863)	4	2.6	>0.05
7	Shigella	PB 1246 \pm 530	12919 (9344–17 862)	5	3.4	>0.05
		SF 13 837 \pm 1600	1928 (1444–2576)	4	6.5	>0.05
	CT	PB 470 \pm 430	> 137 000 —	—	—	—
		SF 1493 \pm 212	36 619 (22 428–59 791)	6	4.8	>0.05

CT, *Chlamydia trachomatis*; CI, confidence intervals; P, probability of single-hit kinetics; No. lev, number of informative cell concentration levels; MNC, mononuclear cells; PB, peripheral blood; SF, synovial fluid.

The specific antigen responses in SF are in bold type.

* Results are expressed as incremental ct/min.

all LDA experiments, APC were derived only from the PB; thus differences in the LDA can be attributed only to differences between the SF and PB non-adherent cells, which are mainly T cells. LDA were carried out in five out of the seven responder patients and four non-responder patients. As can be seen from Table 2, in all cases the results of the LDA were consistent with single-hit kinetics.

T cell frequencies to specific and irrelevant antigens in PB and SF of responder patients. The LDA results for the responder patients are shown in Table 2 and Fig. 2. The frequency of antigen-specific T cells varied widely; the frequency of T cells responding to the specific antigen ranged from 1/619 to 1/4846 (mean 1/2389) in SF and 1/1286 to 1/16 279 (mean 1/7570) in PB whilst the frequency of irrelevant antigen-specific T cells was lower, ranging from 1/5708 to 1/148 888 in SF and 1/3990 to less than 1/215 000 in PB. However, two important general conclusions can be drawn for all patients except patient 2. First, for each individual patient, the frequency of T cells responding to the specific antigen was significantly greater than the frequency of irrelevant antigen-specific T cells in SF; in patients 1 and 7 there was also a major difference in the blood. Second, there was a significantly higher frequency of T cells responding to the specific antigen in the SF compared with PB (Fig. 2). Thus, in the majority of patients (patients 1, 4, 6, 7), the enhanced proliferative response of synovial MNC to the triggering antigen can be explained by an increased frequency of triggering antigen-specific T cells in the joint. In contrast, in patient 2, who had similar PB and SF T cell frequencies to specific and

irrelevant antigen, other mechanisms such as T cell hyper-reactivity must be invoked to explain the bulk culture differences.

T cell frequencies in non-responder patients. In non-responder patients PB and SF T cell frequencies to the antigens tested were of a similar order to those seen in responder patients to the irrelevant antigen; in PB they ranged from 1/8438 to less than 1/215 000 (mean 1/88 438) whilst in SF they varied from 1/7837 to less than 1/215 000 (mean 1/85 436) (data not shown).

DISCUSSION

Many investigators have confirmed the initial observations by Ford *et al.* [3] that ReA SF MNC proliferate specifically to the microbe triggering the disease, whereas paired PB cells proliferate much less or not at all [2,5]. The current study investigated the mechanisms underlying this response. We show, first, that the enhanced response of ReA SF MNC to triggering antigen is due to the T cells rather than the APCs and, second, that in most patients the increased SF MNC response is associated with an increased frequency of T cells in SF responding to the specific antigen.

The strongest evidence in favour of a major role for the APC comes from the work of Life *et al.* who showed a strong proliferative response of PB T cells in the presence of antigen if they were combined with SF APC whilst there was only a minimal response if PB T cells were cultured with PB APC or from unseparated PB MNC [13]. The enhanced cytokine

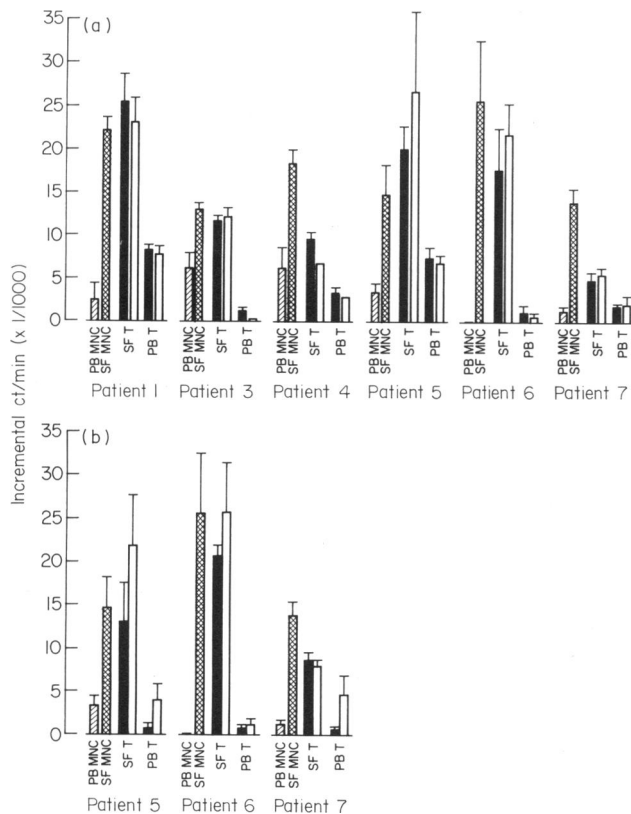


Fig. 1. Proliferation of unseparated mononuclear cells (MNC) and separated T cells (T) from peripheral blood (PB) or synovial fluid (SF) to the triggering bacterium (*Yersinia* in patients 1 and 3, *Chlamydia* in patient 4, *Shigella* in patients 5, 6, 7) in reactive arthritis patients; separation was done either by adherence (a) or by rosetting (b) as described in Patients and Methods. Closed bars, T cells plus antigen presenting cells (APC) from PB; open bars, T cells plus APC from SF.

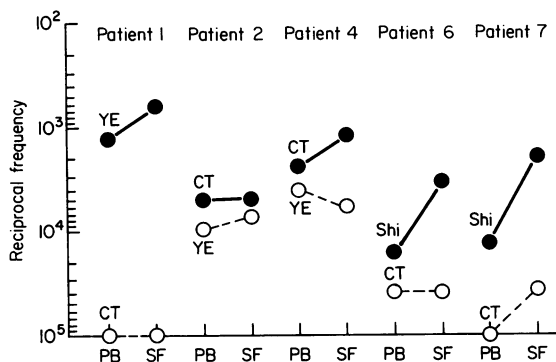


Fig. 2. Frequencies of non-adherent mononuclear cells against triggering (●) or irrelevant bacterium (○) in peripheral blood (PB) or synovial fluid (SF) in reactive arthritis patients. YE, *Yersinia enterocolitica*; CT, *Chlamydia trachomatis*; Shi, *Shigella*.

production of SF APC [11] and the prevalence of dendritic cells among them [12] will render them more effective at supporting antigen-induced lymphocyte proliferation. However, these general mechanisms cannot explain the fact that the enhanced proliferation in ReA is specific for the triggering antigen and not a general phenomenon for all ReA-associated antigens or indeed other recall antigens [10,23]. Furthermore, in other forms of arthritis such as rheumatoid arthritis, similar alterations in APC phenotype and function are seen as in ReA [12,27,28] but there is no enhancement of proliferation to ReA-associated organisms [2,4,10]. The reason for the divergent results of our study and that of Life *et al.* [13] remains unknown. In our initial experiments, we separated T cells and APC by adherence whereas they used rosetting with sheep erythrocytes resulting in APC fractions comprised of different cell types. To exclude this difference, we conducted three experiments using rosetting and adherence methods in parallel and no major difference was found. In particular, the PB T cells did not respond substantially to antigen using either SF adherent cells or non-T cells as APC and certainly not to levels similar to, or even in excess of, SF MNC and SF T cells as described by Life *et al.*

Having shown that the increased synovial T cell proliferation in ReA is mainly related to the T cell and not the APC, we went on to examine whether the enhanced synovial response was related mainly to T cell hyper-reactivity or to an increased frequency of the specific T cells. Because synovial T cells are of the CD45RO⁺ type and activated they are likely to react more rapidly than PB T cells to an antigen even if the T cell specific for that antigen were present at the same frequency in both. It has been suggested that the enhanced SF MNC response can be explained solely on this basis [19,20] with the additional assumption that T cells are recruited into the joint according to their prevalence in the blood. In this hypothesis the specificity of the SF MNC proliferation in ReA would be explained because cells responding to the triggering antigen are present at high frequency in blood due to recent infection whilst the differences between SF and PB would be explained on the basis of the hyper-reactive state of SF T cells. It remains difficult to explain on this basis the results seen in patients where there is no response in the peripheral blood but a very marked proliferation in synovial fluid, for example patients 6 and 7 in our study. It is also hard to understand, using this hypothesis in isolation, why there is not a generally increased response in SF compared with PB for other antigens whose specific T cells are at high frequency in the general circulation such as PPD and TT [10,23]. In summary, whilst the hyper-reactive state of the synovial T cell may be of some relevance, especially in patients such as patient 2 in our study where there is a significant PB response to antigen and where the frequency of antigen-specific T cells does not differ between blood and the joint, it seems most probable that the major mechanism is a selective accumulation of triggering antigen-specific T cells in the joint.

The idea that such T cells might selectively accumulate in the joint is supported by work showing that specific T lymphocytes are selectively recruited into the lung by local antigen [29]; alternatively or in addition, they proliferate *in situ*. In other diseases including leprosy and leishmaniasis [30,31], accumulation of T cells specific for the causative microbe at the site of the lesion has also been demonstrated. More recently the frequency of T cells specific for myelin oligodendrocyte glycoprotein in patients with multiple sclerosis was found to be 1/450

in cerebrospinal fluid and 1/7299 in PB [32] and in tuberculous pleurisy the frequency was 1/2240 in pleural fluid and 1/14 790 in PB [33]. These results bear a close similarity to ours. No previous studies of T cell frequency have been performed in ReA but in the similar arthropathy, Lyme disease, a slightly higher antigen-specific T cell frequency to *B. burgdorferi* in SF compared with PB was found although no direct comparison between SF and PB from the same patients was done [34].

One important point to note is the relatively low frequencies of T cells responding to the specific antigen in the ReA SF with a range from 1/619 to 1/4846. Even assuming that all the specific T cells in a single patient are recognizing the same structure on the triggering organism (by no means certain with a complex microbe) and thus likely to be using the same T cell receptor, it would clearly be difficult to detect oligoclonal T cells at this frequency even using sensitive techniques such as the polymerase chain reaction. This may have important implications for those searching in rheumatoid arthritis and other arthritides where the antigen is unknown.

How accurately can the technique of LDA distinguish between the hyper-reactivity and frequency aspects of the increased response of synovial T cells to triggering antigen? Whilst, because LDA assays are scored on a positive/negative basis rather than on the degree of proliferation, the major element detected by LDA will be increased T cell frequency, presumably there must also be a small threshold effect where hyper-reactive cells at a particular frequency would proliferate rapidly enough to exceed background whereas less activated or naive T cells at the same frequency would not. Also, it remains unclear whether LDA measures the frequency of all antigen-specific T cells or only of hyper-reactive antigen-specific T cells [16,35,36].

Of the three possible explanations for the enhanced response of ReA SF MNC to the triggering bacterial antigen, namely, first, an increased frequency of triggering antigen-specific T cells in the joint, second, the prevalence within the joint of hyper-reactive memory T cells, and, third, a difference between SF and PB APC, the work presented in our study favours a major contribution for the first, a smaller contribution for the second and suggests that the role of the different types of APC is much less significant. Work is currently in progress with triggering antigen-specific T cell clones generated from the synovial fluid of ReA patients to examine the role of the T cell further.

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