Clin Exp Immunol 1993; 94:122–126

Analysis of cytokine profiles in synovial T cell clones from chlamydial reactive arthritis patients: predominance of the Th1 subset

A. K. SIMON*, E. SEIPELT†, P. WU*, B. WENZEL†, J. BRAUN† & J. SIEPER*† *Deutsches RheumaForschungszentrum, and †Department of Medicine, Klinikum Steglitz, Berlin, Germany

(Accepted for publication 15 June 1993)

SUMMARY

Subpopulations of human T cells (Th0, Th1 and Th2) can be distinguished by their cytokine-secretion pattern. Evidence is increasing from other studies that the outcome of a human disease may depend on the subpopulation of T cells that predominates at the site of inflammation. Reactive arthritis serves as a useful model of chronic inflammatory diseases, because the triggering antigen can be identified. Using this triggering antigen we raised 33 T cell clones reactive with *Chlamydia trachomatis* and 25 T cell clones that were not reactive, all from the synovial fluid of two patients suffering from Chlamydia-induced arthritis. Their cytokine secretion patterns for interferon-gamma (IFN-γ), IL-2 and IL-4 were analysed, as also were mRNAs for IFN-γ and IL-10 by *in situ* hybridization. Out of the 33 antigen-reactive clones 23 showed a Th1 pattern with IFN-γ but not IL-4 secretion, while the remaining 10 exhibited a Th0 pattern. The clones that did not react with Chlamydia expressed all patterns of cytokine secretion, including a Th2 pattern, thus providing a control population that excludes bias in the sampling procedure. CD4 and CD8 clones displayed a similar cytokine-secretion pattern. In addition this study demonstrates for the first time the expression of IL-10 mRNA in T cell clones derived from synovial fluid, and this was not confined to the Th2 subset. The Th1 response that Chlamydia provoke can be regarded as appropriate for such an obligate intracellular pathogen.

Keywords reactive arthritis Th1/Th2

INTRODUCTION

Reactive arthritis (ReA) occurs following genito-urinary infection with *Chlamydia trachomatis* or enteral infection with Yersinia, Salmonella, Shigella or Campylobacter [1]. The presence of bacterial antigen in the joint suggests that the immune pathogenesis is driven by persisting antigen [2]. Although the exact cause of pathogenesis is poorly understood, the disease is strongly associated with HLA-B27 [3]. ReA serves as a useful model for other more chronic but even less well understood inflammatory diseases, such as rheumatoid arthritis (RA), because at least the triggering antigen can be identified.

Most of the inflammatory cells in the synovial fluid (SF) of ReA are T cells [4]. They are activated as judged by their CD45RO phenotype [5], and include cells that can proliferate specifically in response to the triggering bacterial antigen [6–8], whereas T cells from the peripheral blood of the same patient show less proliferation. Characterization of antigen-specific T cell clones from the SF therefore provides insight into the role of T cells in the pathogenesis of ReA and possibly other inflammatory disorders.

Two functional lymphokine profiles of T cells can be identified in man [9,10] following observations made originally in the mouse [11]. Th1 cells produce interferon-gamma (IFN-γ) and other lymphokines needed for macrophage activation, thus mediating a protective response to intracellular pathogens, Th2 lymphocytes mediate immunoglobulin class switching and production *via* secretion of IL-4 and IL-6, mobilize eosinophils *via* IL-5, and in addition can down-regulate the Th1 response. In other human inflammatory diseases of infectious origin, such as leprosy [12] and lyme arthritis [13], these Th1 and Th2 lymphokine patterns could be identified in cloned T cells. During infection with *Mycobacterium leprae* a Th1 pattern predominates in the tuberculoid lesions accompanied by a strong cellular response, whereas Th2 cells predominate in lepromatous lesions accompanied by T cell anergy, at the other pole of the disease spectrum [12]. The outcome of the disease may therefore depend on the type of T cell response that predominates. A better understanding of the cytokine patterns could lead to immunotherapy based on cytokines, or on anti-cytokines such as MoAbs or naturally occurring antagonists [14].

Accordingly we have analysed the pattern of lymphokine production of 33 Chlamydia-reactive T cell clones in comparison with 25 T cell clones not reactive with the same antigen. All of them were isolated from the SF of two patients with Chlamydia-induced ReA. Their secretion pattern for IFN-γ, IL-2 and IL-4 was analysed, as also was mRNA for IFN-γ, and for

Correspondence: Katharina Simon, Deutsches Rheumaforschungszentrum, Robert Koch Institut, Nordufer 20, 13353 Berlin, Germany.

the first time mRNA for IL-10 was detected by *in situ* hybridization.

MATERIALS AND METHODS

Patients

Synovial fluid was obtained from two male patients (24 and 31 years old) suffering from acute (less than 3 months of active disease) and self-limited Chlamydia-induced arthritis. Synovial T cells of both patients showed specific proliferation to chlamydial antigen. *C. trachomatis* antigen (CT) was detected in the urogenital swab in one individual who also had IgA and IgG antibodies against CT. The other patient was positive for Chlamydia in a rectal swab and did not show a specific antibody response. Both patients were HLA-B27-positive.

Cell separation, cell culture and proliferation assays

Mononuclear cells (MNC) were separated as previously described [6] from paired samples of peripheral blood and SF by density gradient centrifugation (Lymphoprep, Nycomedas, Norway) and resuspended in tissue culture medium comprising RPMI 1640 (GIBCO, Paisley, UK) with 10% fetal calf serum (FCS; GIBCO), penicillin, streptomycin (100 U/100 μg per ml; Biochrom KG, Berlin, Germany) and glutamine (2 mM/ml; Biochrom KG). Cells were aliquoted into 96-well plates at 10^5 cells/well and stimulated, in triplicates, with the following agents: tissue culture medium alone (background proliferation); *C. trachomatis* (5 μg/ml) grown and purified as described [15]; *Yersinia enterocollitica* (3 μg/ml), grown in trypticase soya bouillon over 48 h and washed in PBS; tetanus toxoid (Behring, Marburg, Germany; 1 μg/ml); pokeweed mitogen (Sigma, Poole, UK; 1 μg/ml). Cells were cultured for 6 days (bulk cultures) or 72 h (clones) at 37°C in 5% CO_2, and ^{3}H-thymidine (Amersham, Aylesbury, UK; 0·2 mCi/well) incorporation was measured as previously described [6].

Cloning procedure

All cloned T cells were isolated from SF-derived MNC that had proved reactive with CT but not to Yersinia or tetanus toxoid antigen in previous proliferation assays. MNC from peripheral blood and SF were stimulated in the presence of normal medium and 5 μg CT antigen for 7 days. Cells were cloned by limiting dilution for each patient in a single event (0·3 cells/well) in Terasaki plates (3000 wells) in the presence of 100 U/ml rIL-2 (Eurocetus GmbH, Frankfurt, Germany), 5 μg/ml phytohaemagglutinin (PHA; Sigma) and 10^6 allogeneic cells irradiated with 40 Gy. Positive clones were transfered from Terasaki plates into 96-well plates, expanded with rIL-2 and restimulated with irradiated allogeneic cells and PHA every 10–14 days. Antigen specificity was tested after stimulation with CT or control antigen in the presence of irradiated (40 Gy) autologous MNC (10 000 T cells to 50 000 autologous MNC). Clones were considered antigen-specific when their stimulation index was above 5 (defined as proliferation with stimulus divided by proliferation without stimulus), and incorporated above 5000 ct/min to a level at least twice as high as that obtained with the control antigen. Two lines mistakenly categorized as clones, but which did not show single TCR-Vβ gene usage (T. Weissensteiner and J. Lanchbury, unpublished results) were subjected to the same cloning procedure a second time.

Analysis of the cloned T cells

Analysis of cells for the expression of surface markers was performed by flow cytometry using a FACScan (Becton Dickinson, Mountain View, CA) with the following MoAbs: Leu-3a (anti-CD4, Becton Dickinson) and Leu-2a (anti-CD8, Becton Dickinson) as previously described [6].

Analysis of soluble lymphokines from the supernatant of T cell clones

The T cell clones were stimulated for lymphokine production at least 5 days after the last expansion with rIL-2 and at least 10 days after the last addition of allogeneic antigen-presenting cells (APC). The cells were washed three times and resuspended in medium at a final concentration of 10^6 cells/ml. They were stimulated either with CT in the presence of 10^6 irradiated autologous APC (clones reactive with CT) or with 3 μg/ml PHA (clones reactive and non-reactive with CT). The supernatant was harvested 20–24 h later and stored at −70°C. After thawing, concentrations of IL-2 and IL-4 were measured with an immuno-enzymatic assay (Quantikine R&D Systems, Minneapolis, MN). IFN-γ was measured by ELISA (Biomar, Marburg, Germany). The minimum detectable dose of IFN-γ and IL-4 is 5 pg/ml, and the sensitivity of the IL-2 assay is 100 pg/ml. According to the manufacturers, the used assays do not demonstrate any cross-reactivity with a variety of other cytokines.

Riboprobes

cRNA probes were prepared by subcloning IFN-γ and IL-10 cDNA fragments into the run-off transcription vector pGEM1 (Promega, Biotec, Madison, WI). All the plasmids used were sequenced. After linearization with appropriate restriction enzymes, antisense (complementary sequence to cytokine mRNA) and sense (identical sequence to cytokine mRNA) probes were generated by transcription, with incorporation of ^{35}S-labelled nucleotides (NEN; Dupont, Bad Homburg, Germany) as described previously [16]. The probe for human IFN-γ was kindly provided by Hermann Herbst (Institut für Pathologie, Klinikum Steglitz, Berlin, Germany), and the human IL-10 cDNA by Cornelia Platzer (Institut für Immunologie, Klinikum Steglitz).

In situ *Hybridization*

In situ hybridization was performed by published methods [16]. Cells (10^5) were cytospun onto pretreated slides (Shandon 2 cytospin centrifuge; Shandon Southern Instruments, Runcorn, UK). The slides were air-dried for 10 min, fixed in 4% paraformaldehyde, and then stored at −70°C up to 8 weeks. For hybridization, cytospun cells were digested with 0·2 N HCl and pronase, followed by acetylation with 0·1 M triethanolamine pH 8·0/0·25% acetic anhydride, and dehydration through graded alcohols. Each slide was hybridized to $2–4 \times 10^5$ ct/min of labelled probe overnight at 50°C. Unhybridized RNA was removed with 50% formamide, followed by RNase-A treatment. Slides were coated with photoemulsion (Amersham LM-1, Amersham, UK), and developed after 8–14 days.

Hybrids between mRNA and cRNA localized as dense collections of silver grains overlaying cells. Negative controls were cytospun preparations hybridized with sense probe (background) and APC alone hybridized with antisense probe. Positive control were cytospun preparations made from COS

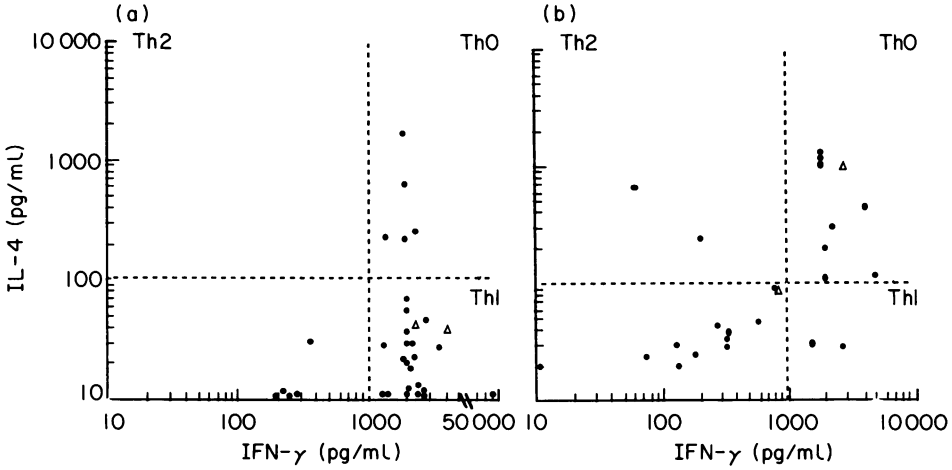

Fig. 1. (a) IL-4 and IFN-γ secretion by *Chlamydia trachomatis*-specific T cell clones derived from synovial fluid after stimulation with chlamydial antigen. ●, CD4 clones; △, CD8 clones. (b) IL-4 and IFN-γ secretion of *C. trachomatis*-non-reactive T cell clones derived from synovial fluid after non-specific stimulation. ●, CD4 clones; △, CD8 clones.

cells transfected with the appropriate expression vector containing the respective cytokine genes [17], kindly provided by Daniel Caput (Sanofi Elf BioRecherches, Labège, France).

RESULTS

Characteristics of T cell clones

The cloning procedure used here yielded 33 CT-specific and 303 CT non-reactive T cell clones. The 33 CT-specific and 25 CT non-reactive T cell clones (randomly selected) were further analysed. The CT-specific clones showed no proliferation with any of the other antigens tested. As mentioned in Materials and Methods, some 'clones' had to be recloned, but this did not appear to perturb the overall picture of cytokine-secretion pattern, since eight subclones which were obtained in the second cloning showed a similar pattern to the clones obtained in the first cloning (data not shown). Most of the 58 SF T cell clones and subclones expressed the CD4 marker (54 out of 58), leaving four out of the total 58 $CD8^+$.

Cytokine-secretion profile

One patient exhibited the Th1 pattern of mainly producing IFN-γ in 20 out of 25 specific clones. The five remaining clones secreted IFN-γ and IL-4, and were therefore assigned to the Th0 category according to the criteria of Mosmann & Coffman [11]. Similarly, the T cell clones (seven out of eight) of the other patient exhibited a Th1 pattern according to the same criteria (Fig. 1a). CT-specific Th1 clones generally showed higher proliferation to chlamydial antigen than CT-specific Th0 clones.

Two CD8 clones were obtained which proved to generate an antigen-specific response according to our criteria (see Materials and Methods). They secreted the Th1 pattern of cytokines. Among the CT-non-reactive clones, the two CD8 clones resembled in cytokine profile the CD4 clones (Fig. 1b). Use of non-viable antigen would account for the paucity of CD8 clones, and the culture conditions may also have been unfavourable. Nine of the antigen-non-reactive T cell clones were of the Th0 type, two of the Th2 type, and two were of the Th1 type, as shown in Fig. 1b.

All clones secreted surprisingly low amounts of IL-2 (only nine clones out of 58 expressed more than 300 pg/ml), and no difference was observed between CT-specific and CT-non-specific clones in the IL-2 secretion.

To test the possibility of culture conditions influencing the pattern of lymphokine secretion as mentioned above, the CT-specific clones were also stimulated with PHA. This non-specific stimulation enhanced IL-2 production equally in all clones, but did not influence significantly the cytokine patterns found among the Chlamydia-specific T lymphocytes (data not shown).

mRNA levels of cytokines

Cytokines were also analysed at the mRNA level to test whether the absence of cytokine protein could be due to consumption. The protein and mRNA data for IFN-γ gave in general concordant results (Fig. 2). Messenger RNA (Fig. 3) was

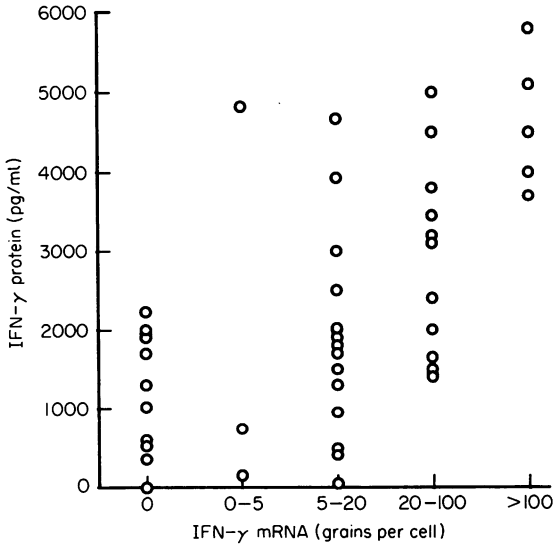

Fig. 2. IFN-γ secretion and mRNA expression of IFN-γ by synovial fluid-derived T cell clones.

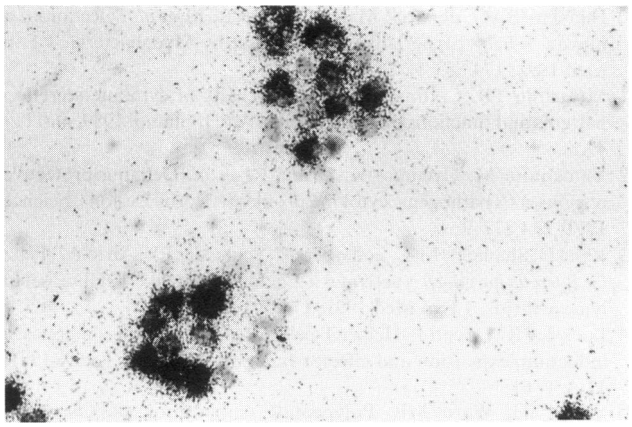

Fig. 3. *Chlamydia trachomatis*-specific T cell clone stimulated with chlamydial antigen hybridized to IFN-γ riboprobe.

present in 33/43 clones, where the protein was also detected in the supernatant. In the remaining protein-secreting clones the mRNA was probably either already degraded at the time of cytospinning because of endogenous RNases, or it fell below the level detectable by *in situ* hybridization.

To exclude that APC secrete cytokines, they were cultured in control experiments with Chlamydia alone for the same period of time (24 h). IL-10 was detected in a few irradiated APC; the amount of grains per cell counted after hybridization was usually five times lower than on those slides where T cell clones and APC were present. This was substracted as background from the signal. All other studied cytokines gave negative results, in supernatants as well as by *in situ* hybridization.

Because MoAb to the human IL-10 was not available, only *in situ* hybridization was performed for this cytokine (Fig. 4). Nine out of 13 specific T cell clones expressed IL-10 mRNA, whereas only one out of eight antigen-non-reactive clones did so. It is unlikely that APC alone account for the hybridization signal, since we did not detect IL-10 in all of the 13 clones tested. Nevertheless, it cannot be ruled out that irradiated APC are able to express IL-10 mRNA in the presence of activated T cells. Three out of 11 T cell clones positive for IL-10 also showed high levels of IL-4, while the remaining eight secreted low amounts of IL-4; IFN-γ expression was equally high in both cell types. Thus IL-10 is evidently not confined to the IL-4-secreting T cell subset.

In any one clone only between 0·1% and 2·5% of all T lymphocytes contained IL-10 mRNA copies (Fig. 4) (half of the cells present on the slide were irradiated APC and were excluded from the cell counting), whereas between 20% and 50% of the clones did so with the IFN-γ riboprobe (Fig. 3). Yet the amount of grains counted per cell was similar for both cytokines.

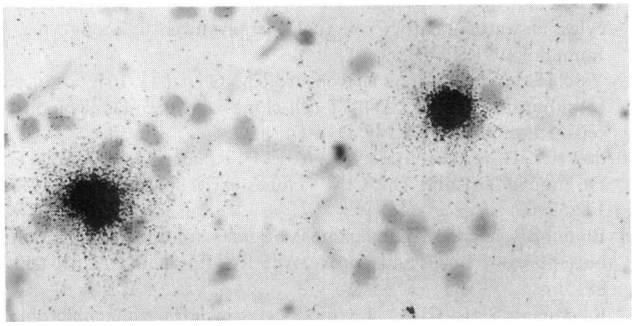

Fig. 4. *Chlamydia trachomatis*-specific T cell clone stimulated with chlamydial antigen hybridized to IL-10 riboprobe.

DISCUSSION

This study shows for the first time that *C. trachomatis*-specific T cell clones isolated from SF exhibit mainly a Th1 pattern, whereas the CT-non-reactive clones exhibit a mixed pattern of cytokine production. These patterns were evident in secreted protein as well as in mRNA. Furthermore, these results demonstrate that SF-derived T cell clones express IL-10 mRNA, regardless of whether they belong to the Th1 or Th2 subset.

Chlamydia are obligate intracellular pathogens living inside macrophages, fibroblasts and epithelial cells [18]. They persist as living organisms in the joint, since chlamydial rRNA [19] can be detected in ReA joints. It is believed that intracellular pathogens characteristically induce a Th1 cell response, with secretion of IFN-γ[20]. Since IFN-γ and tumour necrosis factor-alpha (TNF-α) are the major cytokines responsible for the macrophage activation which results in intracellular killing of Chlamydia [21,22] and since IFN-γ stimulates TNF-α production, a Th1 response as found here can be regarded as appropriate. Other aberrations in the immune response, but not inappropriate production of the cytokines studied here, might therefore explain why only a few individuals develop this form of arthritis. A second possibility is that IFN-γ and consequent TNF-α secretion during the Th1 response, although initially appropriate, might become excessive and damage the joint, as occurs in murine cerebral malaria [23]. However, the present *in vitro* results may not accurately reflect the situation *in vivo* within the synovial membrane. Under different conditions, for instance with a much lower concentration of bacterial antigen in the synovial membrane, the immune response might shift in the Th2 direction. Furthermore, the ReA patients included in this study had the self-limiting form of the disease, and the cytokine pattern could be different in a chronic disease.

The finding of a Th1 response in Chlamydia-induced arthritis is consistent with studies of ReA triggered by other bacteria: *Borrelia burgdorferi* [13] and *Yersinia enterocolitica* [24,25] both selectively induce a Th1 response in the arthritic joint. The presence of a mixed pattern among the non-reactive clones which do not proliferate to CT antigen shows that the Th1 response is not due to bias of the cloning procedure [26]. The addition of IL-2 to the cell culture did not bias the pattern, since IFN-γ and IL-4 were each secreted by some of the cloned T cells.

This is the first report of SF cells expressing IL-10 mRNA. Murine IL-10 is a Th2-type cytokine credited with major inhibitory activity on Th1 cytokine synthesis [27]. Although IL-10 can be secreted by macrophages as well as by T cells [28,29], in the SF investigated here IL-10 is mostly expressed by T lymphocytes. The CT-specific Th1 clones found here secrete high amounts of IFN-γ and almost no IL-4, some also express message for the inhibitory cytokine IL-10. Presumably IL-10 down-regulates Th1 development later in the immune response [29]. Evidently transcription of human IL-10 is not restricted to the Th2 subset [29], and is therefore not as differentially expressed in the human as it is in the mouse [30].

In addition to CD4 clones, we were able for the first time to investigate four CD8 clones from SF; they exhibit a pattern similar to that of the CD4 clones. A classification of CD8 cells according to their secretion pattern might be important, as it has been suggested by Bloom *et al.* [31] that CD8 clones producing IFN-γ can act as cytotoxic cells, whereas those secreting IL-4 exhibit a suppressive effect. Further analysis of CD8 T cells will also gain more relevance in the future because of the known link of the MHC allele HLA-B27 and ReA. Furthermore, there is increasing evidence that CD8 T cells play a major role in the cellular immune response against intracellular organisms [32].

T cells present in the SF are believed to play an important role in the pathogenesis of ReA. Chlamydia-specific T cell clones secrete mainly a Th1 pattern of cytokines, while also secreting IL-10. The data cannot be explained by a bias in the cloning procedure, because this pattern was not detected from non-specific clones isolated in parallel. Caution should be exercised in extrapolating from the data to future cytokine (or anti-cytokine) therapy, because, first, results can be biased by *in vitro* conditions, and second, they take no account of events within the synovial membrane where most of the immunopathology occurs. Further work is needed using *in situ* hybridization combined with immunohistology [9] or polymerase chain reaction [12] and comparing acute self-limiting courses with chronic reactive arthritis.

ACKNOWLEDGMENTS

We are grateful to N. A. Mitchison, Deutsches RheumaForschungszentrum Berlin, for advice and support. This work was supported by the Bundesministerium für Forschung und Technologie, and the Deutsche Forschungsgemeinschaft, Germany.

REFERENCES

1 Keat A. Reiter's syndrome and reactive arthritis in perspective. N Engl J Med 1983; **309**:1606–15.
2 Keat A, Thomas B, Dixey J, Osborn M, Sonnex C, Taylor-Robinson D. Chlamydia trachomatis and reactive arthritis: the missing link. Lancet 1987; **i**:72–74.
3 Brewerton DA, Caffrey M, Nicholls A, Walters D, Oates JK, James DCO. Reiter's disease and HLA B27. Lancet 1973; **ii**:996–8.
4 Sieper J, Braun J, Wu P, Kingsley G. Alteration in T cell/macrophage ratio may reveal lymphocyte proliferation specific for the triggering antigen in reactive arthritis. Scand J Immunol 1992; **36**:427–34.
5 Pitzalis C, Kingsley GH, Covelli M, Meliconi R, Markey A, Panayi GS. The preferential accumulation of helper-inducer T lymphocytes in inflammatory lesions: evidence for regulation by selective endothelial and homotypic adhesion. Eur J Immunol 1988; **18**:1397–404.
6 Sieper J, Kingsley G, Palacois-Boix A *et al.* Synovial T lymphocyte specific immune response to *Chlamydia trachomatis* in Reiter's disease. Arthritis Rheum 1991; **34**:588–98.
7 Sieper J, Braun J, Brandt J *et al.* Pathogenetic role of Chlamydia, Yersinia and Borrelia in undifferentiated oligoarthritis. J Rheumatol 1992; **19**:1236–42.
8 Sieper J, Braun J, Wu P, Kingsley G. T cells are responsible for the enhanced synovial cellular immune response to triggering antigen in reactive arthritis. Clin Exp Immunol 1993; **91**:96–102.
9 Kay AB, Ying S, Varney V *et al.* Messenger RNA expression of the cytokine gene cluster, interleukin (IL-3), IL-4, IL-5, and granulocyte/macrophage colony-stimulating factor, in allergen-induced late-phase cutaneous reactions in atopic subjects. J Exp Med 1991; **173**:775–8.
10 Haanen JBAG, de Waal Malefyt R, Res PCM *et al.* Selection of a human T helper type 1-like T cell subset by Mycobacteria. J Exp Med 1991; **174**:583–92.
11 Mosmann TR, Coffman RL. Heterogeneity of cytokine secretion patterns and functions of helper T cells. Adv Immunol 1989; **46**:111–47.
12 Yamamura M, Uyemura K, Deans RJ *et al.* Defining protective responses to pathogens: cytokine profiles in leprosy lesions. Science 1991; **254**:277–9.
13 Yssel H, Shanafelt M-C, Soderberg C, Schneider PV, Anzola J, Peltz G. *Borrelia burgdorferi* activates a T helper type 1-like T cell subset in lyme arthritis. J Exp Med 1991; **174**:593–601.
14 Locksley RM, Scott P. Helper T-cell subsets in mouse leishmaniasis: induction, expansion and effector function. Immunol Today 1991; **12**:A58–61.
15 Salari SH, Ward ME. Polypeptide composition of *Chlamydia trachomatis*. J Gen Microbiol 1981; **123**:197–207.
16 Milani S, Herbst H, Schuppan D, Hahn EG, Stein H. *In situ* hybridisation for procollagen types I, III, IV mRNA in normal and fibrotic rat liver. Evidence for predominant expression in non-parenchymal liver cells. Hepatol 1989; **10**:84–92.
17 Brunner MC, Caput D, Helbert MR *et al.* Regulation of regulatory T cells. In: Proc 8th Int Congr Immunol, Budapest; Berlin: Springer Verlag, 1992
18 Ward ME. Chlamydial classification, development and structure. Br Med Bull 1983; **39**:109–15.
19 Rahman MU, Cheema MA, Schumacher HR, Hudson AP. Molecular evidence for the presence of Chlamydia in the synovium of patients with Reiter's syndrome. Arthritis Rheum 1992; **35**:521–9.
20 Scott P, Kaufmann SHE. The role of T-cell subsets and cytokines in the regulation of infection. Immunol Today 1991; **12**:346–8.
21 Byrne GI, Schobert CS, Williams DM, Krueger DA. Chracterization of gamma interferon-mediated cytotoxicity to Chlamydia-infected fibroblasts. Infect Immun 1989; **57**:870–4.
22 Shemer-Avni Y, Wallach D, Sarov I. Inhibition of *Chlamydia trachomatis* growth by recombinant tumor necrosis factor. Infect Immun 1988; **56**:2503–6.
23 Grau GE, Fajardo LF, Piguet PF, Allet B, Lambert PH, Vassali P. Tumor necrosis factor as an essential mediator in murine cerebral malaria. Science 1987; **237**:1210–2.
24 Lahesmaa R, Yssel H, Batsford S *et al.* *Yersinia enterolitica* activates a T helper type 1-like T cell subset in reactive arthritis. J Immunol 1992; **148**:3079–85.
25 Schlaak J, Hermann E, Ringhoffer M *et al.* Predominance of Th1-type T cells in synovial fluid of patients with Yersinia-induced reactive arthritis. Eur J Immunol 1992; **22**:2771–6.
26 Maggi E, Parronchi P, Manetti R *et al.* Reciprocal regulatory effects of IFNγ and IL-4 on the *in vitro* development of human Th1 and Th2 clones. J Immunol 1992; **148**:2142–7.
27 Mosmann TR, Moore KW. The role of IL-10 in crossregulation of Th1 and Th2 responses. Immunol Today 1991; **12**:A49–53.
28 de Waal Malefyt R, Abrams J, Bennett B, Figdor CG, de Vries JE. Interleukin 10 (IL-10) inhibits cytokine synthesis by human monocytes: an autoregulatory role of IL-10 produced by monocytes. J Immunol 1991; **174**:1209–20.
29 Yssel H, deWaal Malefyt R, Roncarolo M-G *et al.* IL-10 is produced by subsets of human CD4$^+$ T cell clones and peripheral blood T cells. J Immunol 1992; **149**:2378–84.
30 de Vries JE, de Waal Malefyt R, Yssel H, Roncarolo M-G, Spits H. Do human Th1 and Th2 CD4$^+$ clones exist? Res Immunol 1991; **142**:59–63.
31 Bloom BR, Modlin RL, Salgame P. Stigma variations: observations on suppressor T cells and leprosy. Ann Rev Immunol 1992; **10**:453–88.
32 Kaufmann SHE. CD8$^+$ T lymphocytes in intracellular microbial infections. Immunol Today 1988; **9**:168–74.