Th1/Th17 Cytokine Profiles in Patients with Reactive Arthritis/Undifferentiated Spondyloarthropathy

RAJEEV SINGH, AMITA AGGARWAL, and RAMNATH MISRA

ABSTRACT. ***Objective.*** Data on synovial fluid (SF) cytokine concentrations in patients with reactive arthritis (ReA) or undifferentiated spondyloarthropathy (uSpA) are limited and contradictory. We measured levels of several proinflammatory and immunoregulatory cytokines in SF and sera from patients with ReA/uSpA.

Methods. Interleukin 17 (IL-17), IL-6, interferon-γ (IFN-γ), and IL-12p40, and immunoregulatory cytokines IL-10 and transforming growth factor-ß (TGF-ß) were assayed using ELISA in SF specimens from 51 patients with ReA/uSpA (ReA 21, uSpA 30), 40 patients with rheumatoid arthritis (RA), and 11 patients with osteoarthritis (OA). IL-17, IL-6, IFN-γ, and IL-10 levels were also measured in paired sera samples from patients with ReA/uSpA.

Results. SF concentrations of IL-17, IL-6, TGF-ß, and IFN-γ were significantly higher in patients with ReA/uSpA as compared to RA patients (for IL-17 median 46 pg/ml, range < 7.8–220 vs median < 7.8 pg/ml, range < 7.8–136, $p < 0.05$; for TGF-ß median 4.2 ng/ml, range 1.32–12 vs median 3.01 ng/ml, range 0.6–9.6, $p < 0.01$; for IL-6 median 58 ng/ml, range 2–540 vs median 34.5 ng/ml, range < 0.009–220, $p < 0.05$; for IFN-γ median 290 pg/ml, range < 9.4–1600 vs median 100 pg/ml, range < 9.4–490, $p < 0.05$). SF levels of IL-10 were comparable but the ratio of IFN-γ/IL-10 was significantly higher in ReA/uSpA patients than RA patients (median 3.18, range 0.06–200 for ReA/uSpA vs median 1.0, range 0.03–26.9 for RA; $p < 0.05$). IL-17, IL-6, IL-10, and IFN-γ SF levels were significantly higher than paired serum levels in ReA/uSpA patients ($p < 0.01$ for IL-17, $p < 0.0001$ for IL-6, $p < 0.0001$ for IL-10, and $p < 0.001$ for IFN-γ).

Conclusion. Increased IL-17, IL-6, TGF-ß, and IFN-γ concentrations in ReA/uSpA than in RA suggest that Th1 and Th17 cells could be the major agents in inflammation in ReA/uSpA. (First Release Oct 15 2007; J Rheumatol 2007;34:2285–90)

Key Indexing Terms:
BACTERIA INDUCED SYNOVIAL FLUID CYTOKINES INTERLEUKIN 6
TRANSFORMING GROWTH FACTOR-ß INTERFERON-γ

Reactive arthritis (ReA)/undifferentiated spondyloarthropathy (uSpA) is an inflammatory joint disorder, triggered by infection either of the urogenital tract with *Chlamydia trachomatis*, or of the enteric tract with *Yersinia*, *Salmonella*, *Shigella*, or *Campylobacter*. ReA/uSpA is often a self-limiting disease, but 20% of patients with ReA develop chronic arthritis[1]. Although T cells play a crucial role in pathogenesis of ReA, the exact mechanism of joint damage is unclear[2,3]. It is likely that cytokines play a critical role in the pathogenesis of arthritis. A predominant Th1 cytokine profile, i.e., high concentrations of interferon-γ (IFN-γ) and low concentrations of interleukin 4 (IL-4), has been reported[4–8]. In contrast to the above reports other studies have shown relative predominance of immunosuppressive cytokines IL-4 and IL-10 and relative lack of IFN-γ and tumor necrosis factor-α (TNF-α) in the synovial membrane[9,10] and synovial fluid (SF)[10,11] of ReA patients compared with rheumatoid arthritis (RA).

Plasma transforming growth factor-ß (TGF-ß) levels are higher in ReA as compared to RA[12]. An elevated number of IL-10 and TGF-ß-secreting T cells, B cells, and macrophages were found in the synovial membrane of patients with ReA compared to RA[13]. Yin, *et al*[11] proposed a crucial role of the IL-10/IL-12 balance in the regulation of the cytokine pattern in the joints of patients with ReA. High levels of the inflammatory cytokine IL-6 were also reported in plasma and SF of patients with ReA[6,12].

Thus, data on cytokine concentrations in SF from patients with ReA/uSpA are limited and contradictory, based on studies with small sample sizes. Such findings, however, are important as they provide valuable information on key mediators of inflammation. We investigated the concentrations of several proinflammatory cytokines, IL-17, IL-6, IFN-γ, and IL-12p40 and immunosuppressive cytokines IL-10 and TGF-ß, in a large sample of SF specimens obtained from patients with ReA/uSpA.

From the Department of Immunology, Sanjay Gandhi Post-Graduate Institute of Medical Sciences, Lucknow, India.

R. Singh is a recipient of a Senior Research Fellowship of the Council of Scientific and Industrial Research of India.

R. Singh, PhD Student, MSc; A. Aggarwal, DM, Associate Professor of Clinical Immunology; R. Misra, MD, Professor of Clinical Immunology.

Address reprint requests to Dr. R. Misra, Department of Immunology, Sanjay Gandhi Post-Graduate Institute of Medical Sciences, Lucknow 226014, India. E-mail: rnmisra@sgpgi.ac.in

Accepted July 20, 2007.

MATERIALS AND METHODS

Patients. Fifty-one patients with a diagnosis of ReA/uSpA who presented with acute arthritis of the knee joint with effusion and were considered for therapeutic aspiration/intraarticular corticosteroid administration were included in the study. All patients were seen at the clinical immunology clinic in our tertiary care hospital between November 2003 and December 2005. With patients' consent, 5 ml of blood and 10–20 ml SF were collected at the time of clinical assessment. ReA was defined clinically by the typical clinical picture of acute-onset asymmetrical oligoarthritis of the lower limb joints preceded by a history of diarrhea within 4 weeks. Patients with uSpA were selected if they fulfilled the European Spondylarthropathy Study Group criteria[14].

SF specimens obtained from 40 patients with RA were used as inflammatory disease controls and 11 patients with osteoarthritis (OA) were used as noninflammatory controls.

Determination of cytokines in SF and serum. SF was centrifuged (2000 rpm, 10 min) to remove cellular debris. Sera and SF were stored at –80°C in aliquots until analysis. The sample was thawed only once to avoid degradation. All cytokine levels were determined with commercial ELISA kits according to the manufacturers' instructions. IL-10, IL-12p40, and IFN-γ ELISA were done using Opt EIA kits (BD Biosciences, San Diego, CA, USA), IL-6 and TGF-ß1 using Duo-Set kits (R&D Systems, Minneapolis, MN, USA), and IL-17 using Ready-Set-Go kits (eBioscience, San Diego, CA, USA). IFN-γ, IL-10, IL-6, and IL-17 were analyzed in serum and SF, TGF-ß1 and IL-12p40 were determined only in SF. When sufficient SF was available, assays were performed in duplicate, otherwise they were done singly either neat or diluted with phosphate buffered saline (pH 7.2) 1:1 for IFN-γ, IL-10, and IL-12p40; 1:6 to 1:12 for TGF-ß1; and 1:50 to 1:400 for IL-6. Neat sera were used for detection of IFN-γ, IL-10, IL-6, and IL-12p40. The minimum detection limits of the assays were as follows: IL-17 and IL-10, 7.8 pg/ml; TGF-ß1 and IL-12p40, 31.3 pg/ml; and IL-6 and IFN-γ, 9.4 pg/ml.

Statistical analysis. All data are shown as median (range). Statistical analyses were performed using SPSS version 9 software (SPSS, Chicago, IL, USA). Comparison between groups was by Mann-Whitney 2-tailed test and Wilcoxon signed-rank test. Spearman's test was used for correlation analysis.

RESULTS

Among the 51 patients with ReA/uSpA, 21 had preceding history of diarrhea. Demographic details of patients are given in Table 1.

SF cytokine levels of TGF-ß, IL-6, IL-17, IL-10, IFN-γ, and IL-12p40 were found to be significantly higher in patients with ReA/uSpA as compared to OA (Table 2), whereas TGF-ß, IL-6, IL-17, and IFN-γ concentrations were higher in patients with ReA/uSpA than in RA patients ($p < 0.05$ for TGF-ß, $p < 0.05$ for IL-6, $p < 0.05$ for IL-17, and $p < 0.05$ for IFN-γ; Figure 1). SF levels of IL-10 and IL-12p40 were not significantly different between patients with ReA/uSpA and those with RA (Table 2). The IFN-γ/IL-10 ratio was significantly higher in ReA/uSpA patients as compared to patients with RA ($p < 0.05$), while there was no significant difference between ReA and RA as far as IL-12/IL-10 ratio was concerned (Table 2).

IL-17, IL-6, IL-10, and IFN-γ SF levels were significantly higher than paired serum levels (median 26 pg/ml, range < 9.4–700 for IL-6; median < 9.4 pg/ml, range < 9.4–340 for IFN-γ; median < 7.8 pg/ml, range < 7.8–125 for IL-10; below detectable limit in all serum samples for IL-17) in ReA/uSpA patients ($p < 0.01$ for IL-17, $p < 0.002$ for IL-6, $p < 0.0001$ for IL-10, and $p < 0.001$ for IFN-γ; Figure 2). TGF-ß and IL-12p40 determinations were not done in sera due to paucity of samples. No significant differences of the 7 cytokines tested were found between HLA-B27-positive ($n = 38$) and HLA-B27-negative ($n = 13$) ReA/uSpA patients' SF levels.

A positive correlation was found between SF TGF-ß and IL-6 levels ($r = 0.46$, $p < 0.01$), SF IL-17 and IL-6 levels ($r = 0.62$, $p < 0.001$), and SF TGF-ß and IL-10 levels ($r = 0.46$, $p < 0.01$).

DISCUSSION

Our data show significantly higher synovial fluid IL-17, TGF-ß, IL-6, and IFN-γ levels in ReA/uSpA as compared to RA, while levels of IL-10 and IL-12p40 were comparable to RA. IL-17, IL-6, IFN-γ, and IL-10 were significantly higher in SF than in serum in patients with ReA/uSpA. The SF IFN-γ/IL-10 ratios were significantly higher in patients with ReA/uSpA than in RA. Further, there was significant correlation of IL-17 with IL-6, and of TGF-ß with IL-6 and IL-10.

Ours is the first study with a large number of SF samples from ReA/uSpA patients that has shown higher IL-17, TGF-ß, and IL-6 levels in these patients as compared to patients with RA. In a previous study where SF from patients with seronegative SpA was compared with RA there were 8 patients with ReA, and no difference was found in the levels of TGF-ß and IL-6 between RA and ReA[6]. Our contrasting

Table 1. Demographic features of patients.

Features	ReA, n = 21	uSpA, n = 30
M:F ratio	18:3	26:4
Median age, yrs (range)	26 (16–53)	27 (15–42)
Median duration of disease, yrs (range)	0.75 (0.1–19)	1.5 (0.1–13)
Median duration of current episode, wks (range)	3.8 (1–17.5)	8.5 (1–104)
Oligoarthritis	21	30
Inflammatory backache	10	16
Enthesitis	2	7
HLA-B27-positive	16	22
Positive family history	1	1

Table 2. Synovial fluid cytokine concentrations in 3 study groups. All values expressed as median (range).

	Reactive Arthritis/ Undifferentiated SpA, n = 51 (in SF)	RA, n = 40 (in SF)	OA, n = 11 (in SF)
TGF-ß, ng/ml	4.2 (1.32–12)*††	3.01 (0.6–9.6)† (n = 38)	1.5 (1.03–4.68) (n = 10)
IL-17, pg/ml	46 (77.8–220)*† (n = 33)	< 7.8 (< 7.8–136) (n = 31)	< 7.8 (< 7.8–84) (n = 10)
IL-10, pg/ml	106 (8–560)†	110 (< 7.6–290)† (n = 33)	< 7.6 (< 7.6–110) (n = 7)
IL-6, ng/ml	58 (2–540)*†† (n = 50)	34.5 (< 0.009–220)† (n = 32)	7.25 (0.33–20) (n = 7)
IL-12p40, pg/ml	360 (< 31.3–1450)† (n = 39)	400 (< 31.3–1360)† (n = 31)	155 (< 31.3–660) (n = 10)
IFN-γ, pg/ml	290 (< 9.4–1600)*† (n = 30)	100 (< 9.4–490) (n = 31)	52 (< 9.4–240) (n = 10)
IFN-γ/IL-10	3.18 (0.06–200)* (n = 30)	1 (0.03–26.9) (n = 31)	ND
IL-12/IL-10	3.33 (0–30.7) (n = 39)	2.7 (0–123.07) (n = 29)	ND

* $p < 0.05$ vs RA patients. † $p < 0.05$, †† $p < 0.0001$ vs OA patients. n: number of samples analyzed if different from 51 for ReA, 40 for RA, and 11 for OA groups. ND: not done.

results could be due to the small sample size, or may be related to the sensitivity of assays used. Immunohistochemistry analysis of synovial tissue has shown increased numbers of TGF-ß-secreting T cells, B cells, and macrophages in patients with ReA compared to RA[13]. TGF-ß is an immunosuppressive cytokine that can downmodulate the effector T cells, resulting in resolution of inflammation[15,16]. Upon activation, naive T helper (Th) cells differentiate into effector cells. Until recently, Th cells were classified into Th1 and Th2, based on their cytokine secretion and immunoregulatory functions[17,18]. Th1 cells secrete IFN-γ and regulate cellular immunity, while Th2 cells produce IL-4, IL-5, and IL-13 and mediate humoral responses. Recently, a third category of IL-17-producing T helper cells (Th17) has been identified[19,20]. Th17 cells play a critical role in immunoinflammatory responses seen in autoimmune diseases such as experimental allergic encephalitis and RA[21-24]. Differentiation of Th0 cells to Th17 cells is mediated by simultaneous presence of TGF-ß and IL-6[25,26]. Our findings of higher levels of IL-17, IL-6, and TGF-ß, with good correlation between their levels, may suggest that in ReA/uSpA this leads to generation of proinflammatory Th17 cells. There are no data to date on IL-17 levels or frequencies of Th17 cells in patients with ReA/uSpA. Recently, it has been shown that Th17 cells have potential for osteoclastogenesis and lead to bone destruction in animal models of arthritis[27]. However, we did not quantify Th17 cells, which would have substantiated our observation of increased levels of IL-17 in SF. It would be interesting to see whether IL-23 levels are increased, as IL-23 is required for maintenance and expansion of committed Th17 effectors[26,28].

Our observation of elevated levels of IFN-γ in ReA/uSpA as compared to RA is in contrast to previous data[9-11]. This difference could be due to the nature of biological samples used, that is, SF mononuclear cells and tissue in earlier studies as compared to SF used in this study. Moreover, the difference may be due to the sensitivity of the technique used, i.e., the tissue immunofluorescence method[10] and *in vitro* cytokine secretion[29] in other studies as compared to ELISA in this work. Using an intracellular cytokine assay, a predominant Th1 profile has been seen in patients with ReA/uSpA[30,31]. Even most T cell clones from ReA patients have a Th1 phenotype[32,33]. In paired samples as well, the SF concentration of IFN-γ in ReA/uSpA was significantly higher than that in serum, suggesting a local Th1 response[4]. Further, the observation of a significantly higher SF IFN-γ/IL-10 ratio in ReA/uSpA than in RA patients in our study substantiates a previous report[34] and supports the observation that the cytokine profile in ReA/uSpA has a Th1 bias. Despite being a Th1-dominant disease, ReA is a self-limiting disease in the majority of cases. The increased level of IFN-γ could be regulating the inflammation caused by Th17 cells, as these cells are inhibited by IFN-γ[19,20,35].

The levels of IL-10 and IL-12 as well as the IL-12/IL-10 ratio in ReA were comparable to those of RA. Previous reports show conflicting results regarding IL-10 and IL-12 levels in ReA synovial fluid compared to RA[6,8,34]. The balance between IL-12 and IL-10 is critical in the immunoregulation of and susceptibility to inflammatory and autoimmune diseases[11,36]. IL-10 can be considered a counterregulator of the effects of IL-12. Indeed, IL-10 downregulates monocyte IL-12 production[37] and abrogates IL-12-driven Th1 responses by decreasing the transcription of the p40

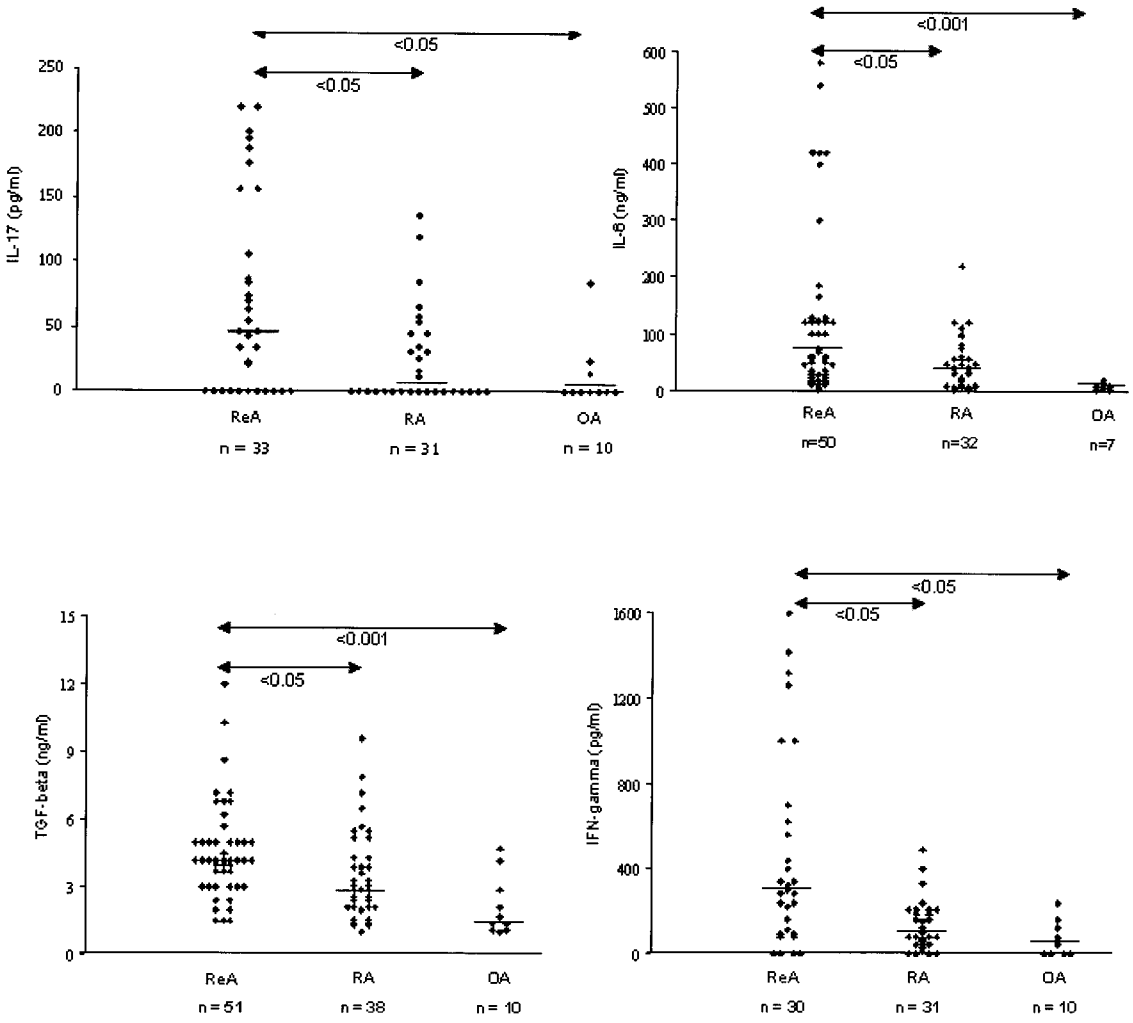

Figure 1. Concentrations of IL-17, IL-6, TGF-ß, and IFN-γ in synovial fluids of patients with ReA, RA, and OA. Number of patients is shown for each group. All intergroup comparisons by Mann-Whitney U-test.

subunit of the IL-12 receptor[38]. Some reports indicate that the higher SF levels of IL-10 observed in ReA and RA[39-42] are still insufficient to control the immunoinflammatory response[43,44]. In other words, the counterbalancing effect of IL-10 on Th1 cytokines in ReA predicted from *in vitro* studies may not be active *in vivo*.

There was no significant difference in the SF levels of IFN-γ and other measured cytokines in HLA-B27-positive than in HLA-B27-negative patients, which could be related to the small number of HLA-B27-negative patients in our study. This is in contrast to earlier studies showing a low level of IFN-γ secretion by peripheral blood mononuclear cells from patients with a long disease duration and presence of HLA-B27[45]. Similarly, in another report, SF IFN-γ levels *in vivo* were significantly lower in HLA-B27-positive patients with *C. trachomatis* ReA[27].

The presence of heterophilic antibodies or autoantibodies such as IgM/IgG/IgA rheumatoid factors (RF) in serum and synovial fluid can give rise to false-positive signals of cytokine detection by ELISA or multiplex immunoassays, requiring neutralization by animal sera or protein-L prior to investigation of the cytokines[46]. In principle, RF could interfere with the detection of cytokines or other biomarkers based on antibody binding such as sandwich ELISA, but generally, neutralization is not done prior to estimation of cytokines, even if samples from patients with RA[34,47] were used in the study. We had not neutralized RF of the synovial fluid. ReA and uSpA patients in this study were seronegative, but we used patients with RA as disease controls. Neutralization of RF in the synovial fluid of patients with RA would have made the difference of cytokine findings between ReA/uSpA even more significant.

Our results from a fairly large number of patients with ReA/uSpA provided a characteristic cytokine profile in these groups distinctive from that of RA. It would be interesting to study Th17 cells in the synovial cells and membranes of these patients with ReA/uSpA.

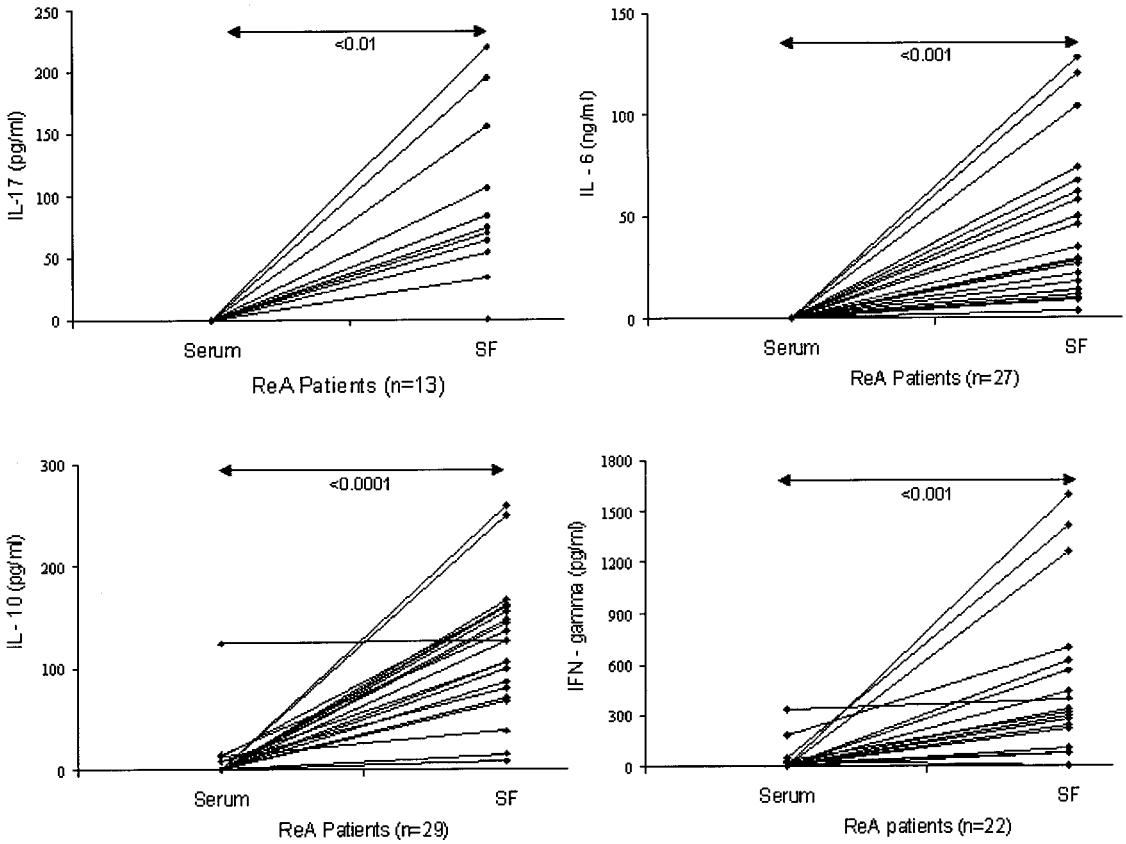

Figure 2. Concentrations of IL-17, IL-6, Il-10, and IFN-γ in synovial fluids (SF) and paired serum samples. Number of patients is shown for each group.

REFERENCES

1. Glennas A, Kvien TK, Melby K, et al. Reactive arthritis: a favorable 2 year course and outcome, independent of triggering agent and HLA-B27. J Rheumatol 1994;21:2274-80.
2. Burmester GR, Daser A, Kamradt T, et al. Immunology of reactive arthritides. Annu Rev Immunol 1995;13:229-50.
3. Sieper J, Braun J. Pathogenesis of spondylarthropathies. Persistent bacterial antigen, autoimmunity, or both? Arthritis Rheum 1995;38:1547-54.
4. Lahesmaa R, Yssel H, Batsford S, et al. Yersinia enterocolitica activates a T helper type 1-like T cell subset in reactive arthritis. J Immunol 1992;148:3079–85.
5. Simon AK, Seipelt E, Wu P, Wenzel B, Braun J, Sieper J. Analysis of cytokine profiles in synovial T cell clones from chlamydial reactive arthritis patients: predominance of the Th1 subset. Clin Exp Immunol 1993;94:122–6.
6. Schlaak JF, Pfers I, Meyer zum Buschenfelde KH, Marker-Hermann E. Different cytokine profiles in the synovial fluid of patients with osteoarthritis, rheumatoid arthritis and seronegative spondylarthropathies. Clin Exp Rheumatol 1996;14:155–62.
7. Kotake S, Schumacher HR Jr, Arayssi TK, et al. Gamma interferon and interleukin-10 gene expression in synovial tissues from patients with early stages of Chlamydia associated arthritis and undifferentiated oligoarthritis and from healthy volunteers. Infect Immun 1999;67:2682–6.
8. Ribbens C, Andre B, Kaye O, et al. Increased synovial fluid levels of interleukin-12, sCD25 and sTNF-RII/sTNF-RI ratio delineate a cytokine pattern characteristic of immune arthropathies. Eur Cytokine Netw 2000;11:669-76.
9. Simon AK, Seipelt E, Sieper J. Divergent T-cell cytokine patterns in inflammatory arthritis. Proc Natl Acad Sci USA 1994;91:8562–6.
10. Yin Z, Siegert S, Neure L, et al. The elevated ratio of interferon gamma-/interleukin-4-positive T cells found in synovial fluid and synovial membrane of rheumatoid arthritis patients can be changed by interleukin-4 but not by interleukin-10 or transforming growth factor beta. Rheumatology Oxford 1999;38:1058–67.
11. Yin Z, Braun J, Neure L, et al. Crucial role of interleukin-10/interleukin-12 balance in the regulation of the type 2 T helper cytokine response in reactive arthritis. Arthritis Rheum 1997;40:1788–97.
12. Claudepierre P, Rymer JC, Authier FJ, et al. A relationship between TGF-ß or IL-6 plasma levels and clinical features of spondyloarthropathies. Br J Rheumatol 1997;36:400-1.
13. Appel H, Neure L, Kuhne M, Braun J, Rudwaleit M, Sieper J. An elevated level of IL-10- and TGF-beta-secreting T cells, B cells and macrophages in the synovial membrane of patients with reactive arthritis compared to rheumatoid arthritis. Clin Rheumatol 2004;23:435-40.
14. Dougados M, van der Linden S, Juhlin R, et al. The European Spondylarthropathy Study Group preliminary criteria for the classification of spondylarthropathy. Arthritis Rheum 1991;34:1218-27.
15. Tiemessen MM, Kunzmann S, Schmidt-Weber CB, et al. Transforming growth factor-beta inhibits human antigen-specific CD4+ T cell proliferation without modulating the cytokine response. Int Immunol 2003;15:1495-504.
16. Song XY, Gu M, Jin WW, Klinman DM, Wahl SM. Plasmid DNA encoding transforming growth factor-beta-1 suppresses chronic disease in a streptococcal cell wall-induced arthritis model. J Clin

Invest 1998;101:2615-21.
17. Mosmann TR, Coffman RL. Th1 and Th2 cells: different patterns of lymphokine secretion lead to different functional properties. Annu Rev Immunol 1989;7:145-73.
18. Dong C, Flavell RA. Cell fate decision: T-helper 1 and 2 subsets in immune responses. Arthritis Res 2000;2:179-88.
19. Park H, Li Z, Yang XO, et al. A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17. Nat Immunol 2005;6:1133-41.
20. Harrington LE, Hatton RD, Mangan PR, et al. Interleukin 17-producing CD4+ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. Nat Immunol 2005;6:1123-32.
21. Cua DJ, Sherlock J, Chen Y, et al. Interleukin-23 rather than interleukin-12 is the critical cytokine for autoimmune inflammation of the brain. Nature 2003;421:744–8.
22. Kolls JK, Linden A. Interleukin-17 family members and inflammation. Immunity 2004;21:467–76.
23. Langrish CL, Chen Y, Blumenschein WM, et al. IL-23 drives a pathogenic T cell population that induces autoimmune inflammation. J Exp Med 2005;201:233–40.
24. Hirota K, Hashimoto M, Yoshitomi H, et al. T cell self-reactivity forms a cytokine milieu for spontaneous development of IL-17+ Th cells that cause autoimmune arthritis. J Exp Med 2007;204:41-7.
25. Veldhoen M, Hocking RJ, Atkins CJ, Locksley RM, Stockinger B. TGF-beta in the context of an inflammatory cytokine milieu supports de novo differentiation of IL-17-producing T cells. Immunity 2006;24:179-89.
26. Bettelli E, Carrier Y, Gao W, et al. Reciprocal developmental pathways for the generation of pathogenic effector Th17 and regulatory T cells. Nature 2006;441:235–38.
27. Sato K, Suematsu A, Okamoto K, et al. Th17 functions as an osteoclastogenic helper T cell subst that links T cell activation and bone destruction. J Exp Med 2006;203:2673-82.
28. Weaver CT, Harrington LE, Mangan PR, Gavrieli M, Murphy KM. Th17: an effector CD4 T cell lineage with regulatory T cell ties. Immunity 2006;24:677–88.
29. Butrimiene I, Jarmalaite S, Ranceva J, Venalis A, Jasiuleviciute L, Zvirbliene A. Different cytokine profiles in patients with chronic and acute reactive arthritis. Rheumatology Oxford 2004;43:1300-4.
30. Thiel A, Wu P, Lauster R, Braun J, Radbruch A, Sieper J. Analysis of the antigen-specific T cell response in reactive arthritis by flow cytometry. Arthritis Rheum 2000;43:2834-42.
31. Saxena S, Aggarwal A, Misra R. Outer membrane protein of Salmonella is the major antigenic target in patients with Salmonella induced reactive arthritis. J Rheumatol 2005;32:86–92.
32. 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.
33. Simon AK, Seipelt E, Wu P, Wenzel B, Braun J, Sieper J. Analysis of cytokine profiles in synovial T cell clones from chlamydial reactive arthritis patients: predominance of the Th1 subset. Clin Exp Immunol 1993;94:122-6.
34. Bas S, Kvien TK, Buchs N, Fulpius T, Gabay C. Lower level of synovial fluid interferon-γ in HLA-B27-positive than in HLA-B27-negative patients with Chlamydia trachomatis reactive arthritis. Rheumatology Oxford 2003;42:461-67.
35. Cruz A, Khader SA, Torrado E, et al. Cutting edge: IFN-γ regulates the induction and expansion of IL-17 producing CD4 T cells during mycobacterial infection. J Immunol 2006;177:1416-20.
36. Segal BM, Dwyer BK, Shevach EM. An interleukin (IL)-10/IL-12 immunoregulatory circuit controls susceptibility to autoimmune disease. J Exp Med 1998;187:537-46.
37. D'Andrea A, Aste-Amezaga M, Valiante NM, Ma X, Kubin M, Trinchieri G. Interleukin-10 inhibits human lymphocyte interferon-gamma production by suppressing natural killer cell stimulatory factor/IL-12 synthesis in accessory cells. J Exp Med 1993;178:1041-8.
38. Aste-Amezaga M, Ma X, Sartori A, Trinchieri G. Molecular mechanisms of the induction of IL-12 and its inhibition by IL-10. J Immunol 1998;160:5936-44.
39. Bucht A, Larsson P, Weisbrot L, et al. Expression of interferon-gamma, IL-10, IL-12 and transforming growth factor-beta mRNA in synovial fluid cells from patients in the early and late phases of rheumatoid arthritis. Clin Exp Immunol 1996;103:357-67.
40. Cush JJ, Splawski JB, Thomas R, et al. Elevated interleukin-10 levels in patients with rheumatoid arthritis. Arthritis Rheum 1995;38:96-104.
41. Lapadula G, Iannone F, Dell'Accio F, Covelli M, Pipitone V. Interleukin-10 in rheumatoid arthritis. Clin Exp Rheumatol 1995;13:629-32.
42. Isomaki P, Luukkainen R, Saario R, Toivanen P, Punnonen J. Interleukin-10 functions as an antiinflammatory cytokine in rheumatoid synovium. Arthritis Rheum 1996;39:386-95.
43. Keystone E, Wherry J, Grint P. IL-10 as a therapeutic strategy in the treatment of rheumatoid arthritis. Rheum Dis Clin North Am 1998;24:629-39.
44. Brennan FM. Interleukin 10 and arthritis. Rheumatology Oxford 1999;38:293-7.
45. Braun J, Yin Z, Spiller I, et al. Low secretion of tumor necrosis factor alpha, but no other Th1 or Th2 cytokines, by peripheral blood mononuclear cells correlates with chronicity in reactive arthritis. Arthritis Rheum 1999;42:2039-44.
46. de Jager W, Rijkers GT. Solid-phase and bead-based cytokine immunoassay: a comparison. Methods 2006;38:294-303.
47. van Amelsfor JM, van Roon JA, Noordegraaf M, et al. Proinflammmatory mediator-induced reversal of CD4+,CD25+ regulatory T cell mediated suppression in rheumatoid arthritis. Arthritis Rheum 2007;56:732-42.