Interleukin 17 Levels Are Increased in Juvenile Idiopathic Arthritis Synovial Fluid and Induce Synovial Fibroblasts to Produce Proinflammatory Cytokines and Matrix Metalloproteinases

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ABSTRACT. ***Objective.*** Cytokines are the major mediators of joint damage in chronic arthritis. Data on synovial fluid (SF) concentration of Th17 cell-derived cytokine interleukin 17 (IL-17) in patients with juvenile idiopathic arthritis (JIA) are sparse. We measured levels of IL-17 in SF specimens from children with enthesitis-related arthritis (ERA) and polyarticular JIA (poly-JIA), and studied the ability of IL-17 to produce matrix metalloproteinases (MMP) and cytokines by fibroblast-like synoviocytes (FLS) from patients with ERA.

Methods. IL-17 levels were measured in SF of patients with ERA (n = 43), poly-JIA (n = 17), rheumatoid arthritis (RA; n = 35), and osteoarthritis (OA; n = 10) by ELISA. In patients with JIA, 10 paired serum samples were also assayed. FLS were cultured from SF of patients with ERA and subsequently stimulated for 48 h by IL-17 or tumor necrosis factor-α. Later the production of IL-6, IL-8, MMP-1, MMP-3, and tissue inhibitor of metalloproteinase (TIMP)-1 was measured in the culture supernatants by ELISA.

Results. Median IL-17 levels in SF were higher in patients with JIA [28 pg/ml (range 0–200)] compared to OA [0 pg/ml (range 0–84); $p < 0.001$] and RA ($p < 0.05$). The levels were comparable between poly-JIA patients and the ERA group. The median SF IL-17 levels were significantly higher compared to serum levels in children with JIA ($p < 0.005$). In ERA, SF IL-17 correlated with number of swollen joints ($r = 0.35$; $p < 0.05$), number of joints with limited mobility ($r = 0.55$; $p < 0.001$), and number of tender joints ($r = 0.46$; $p < 0.01$); however, no correlation was seen with erythrocyte sedimentation rate. IL-17 induced FLS to produce IL-6, IL-8, MMP-3, and MMP-1. However, there was no effect on the production of TIMP.

Conclusion. Increased IL-17 levels in ERA SF correlate with disease activity and this may be due to increased production of MMP and cytokines by IL-17. (First Release Jan 15 2008; J Rheumatol 2008;35:515–9)

Key Indexing Terms:
CYTOKINES JUVENILE RHEUMATOID ARTHRITIS TH17 CELLS INFLAMMATION

Juvenile idiopathic arthritis (JIA) is a chronic arthritis of childhood characterized by synovitis, cartilage destruction, and periarticular bone resorption. The synovium is infiltrated by T lymphocytes and macrophages, and mediators released from these cells such as proinflammatory cytokines, chemokines, and degradative enzymes cause cartilage and bone destruction[1]. Proteolytic enzymes like matrix metalloproteinases (MMP) play an important role in cartilage and bone loss. The activity of MMP is regulated by its natural inhibitor, tissue inhibitor of metalloproteinase (TIMP). Enhanced expression of MMP and low expression of TIMP-1 is seen in the lining layer of synovial tissue in JIA[2]. Increased levels of MMP-3 and MMP-1 are present in synovial fluid (SF) of patients with JIA[3,4]. The relative expression of TIMP versus MMP in joint tissue alters the balance between the maintenance of articular cartilage and its destruction in diseases such as RA and JIA. Cytokines are involved in many aspects of inflammation, including leukocyte recruitment, production of MMP, and other proinflammatory mediators in the joint. Interleukein 1 (IL-1) and tumor necrosis factor (TNF) preferentially induce MMP-3 and MMP-1 expression, but not TIMP expression, and thus enhance cartilage degradation[5].

Recent evidence suggests that IL-17-producing Th17 cells have a crucial role in autoimmune inflammation[6]. IL-17 can induce production of IL-6 and IL-8 by fibroblasts[7]. IL-17 is of interest as it is similar to TNF-α and IL-1, and may have effect

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on MMP production[8]. The role of IL-17 in JIA remains to be studied. The IL-23–IL-17 axis is critical not only for the onset phase, but also for the bone destruction phase of autoimmune arthritis[9]. Levels of IL-17 in SF were reported to be significantly higher in patients with rheumatoid arthritis (RA) than in patients with osteoarthritis (OA)[10]. In human *ex vivo* models, addition of IL-17 enhanced IL-6 production and collagen destruction and inhibited collagen synthesis by RA synovium explants[11]. Data from animal models also suggest a role of IL-17 in cartilage degradation. IL-17-deficient mice were resistant to induction of collagen-induced arthritis (CIA)[12]. Joint inflammation and cartilage and bone destruction were suppressed after administration of anti-IL-17 antibody in a CIA model[13].

Data on SF and serum levels of IL-17 in JIA are sparse[14]. In addition, the spectrum of JIA is different in people of the Indian subcontinent, with large numbers of children having enthesitis-related arthritis (ERA)[15]. Therefore we studied the concentrations of IL-17 in SF and serum obtained from a group of children with ERA and polyarticular JIA (poly-JIA). The effect of IL-17 on regulating the expression of MMP-3 and MMP-1 versus TIMP-1 in synovial fibroblast cells isolated from human joint tissues has not been studied. We studied the production of MMP-3, MMP-1, and TIMP-1 from fibroblast-like synoviocytes (FLS) derived from patients with ERA on treatment with recombinant IL-17.

MATERIALS AND METHODS

SF specimens were obtained from patients with JIA (International League of Associations for Rheumatology criteria[16]) after informed consent was obtained. SF specimens obtained from 35 patients with RA were used as disease controls and 10 patients with OA were used as controls. Peripheral blood was collected from 10 patients with JIA and 10 healthy controls. Our study was approved by institutional ethics committee.

SF was centrifuged at 2000 rpm for 10 min to remove cellular debris and was stored at –80°C in aliquots until analysis. Each aliquot was thawed only once to avoid degradation.

SF levels of IL-17, IL-6, IL-1ß, and TNF-α. The levels of IL-17 in SF and serum were measured using ELISA development kits (eBiosciences, San Diego, CA, USA). The minimal detection limit of the IL-17 assay was 4 pg/ml. IL-6, IL-1ß, and TNF-α levels were measured using Duo set ELISA development kits (R&D Systems, Minneapolis, MN, USA). The minimal detection limit of the assay was 4.6 pg/ml for IL-6, 15.6 pg/ml for IL-1ß, and 15.6 pg/ml for TNF-α.

In vitro effect of IL-17 on FLS. Culture of FLS from SF. FLS were generated from 2 patients with RA and 4 patients with ERA as described[17]. SF was collected in heparinized syringes and centrifuged at 2000 rpm for 10 min. The resulting cell pellet was resuspended in 5 ml of minimum essential medium (MEM; Hyclone, Logan, UT, USA) with 15% heat inactivated fetal bovine serum (FBS), 1% nonessential amino acid, and 1% penicillin/streptomycin solution and plated in 25 ml tissue culture flasks. Cells were incubated at 37°C with 5% CO_2 for 24 h. Then medium was aspirated and cells were washed with phosphate buffered saline (PBS) to remove nonadherent cells. Growth medium was replaced every 3 to 4 days. After 10 to 14 days adherent cells were removed from flasks by trypsinization, washed, and split 1:2 in 25 cm^2 flasks. Cells in passages 3 to 6 were used for experiments.

Stimulation assays. Recombinant IL-17 and TNF-α were purchased from BD Pharmingen, San Diego, CA, USA. Cultured synovial fibroblast (10,000 cells per well) were stimulated with 50 ng/ml of IL-17 and 10 ng/ml of TNF-α in 24-well culture plates for 48 h (positive control). Culture supernatant were collected and stored at –80°C for subsequent ELISA experiments.

ELISA. Levels of pro-MMP-1 and MMP-3 in culture supernatant were measured using enzyme immunoassay (Quantikine, R&D Systems). The quantikine MMP-3 immunoassay measures total MMP-3 (pro- and active MMP3).

TIMP and IL-6 levels were measured using Duo set ELISA development kits (R&D Systems) and IL-8 with OptEIA™ ELISA sets (BD PharMingen). The minimum detection limits of the assays were as follows: MMP-3, MMP-1, 0.156 ng/ml; TIMP-1, 31.2 pg/ml; IL-6, 9.3 pg/ml; and IL-8, 3.1 pg/ml.

Statistical analysis. Group data of SF are indicated as median and range. Mann-Whitney U-test was used to compare data between 2 groups. Wilcoxon sign-ranked tests were used for paired data. Pearson's correlation coefficient was used to determine associations between different cytokines and measures of disease activity.

RESULTS

There were 43 patients with ERA (42 male, 1 female) and 17 patients with poly-JIA (13 male, 4 female). In ERA the median age was 14 years (range 3–35), age at onset 12 years (range 4–15), and disease duration 4 years (range 0.5–26). Among these, 29 were HLA-B27-positive and 4 were receiving disease modifying antirheumatic drugs (DMARD).

In poly-JIA the median age was 15 years (range 9–22), age at onset 11 years (range 1–15), and disease duration 6 years (0.5-17). Among these, 4 were receiving DMARD. Among these 17, 4 were rheumatoid factor-positive but none was positive for antinuclear antibodies.

In the RA control group (12 men, 23 women), median age was 45 years (range 24–65) and all were receiving DMARD. The median age of healthy controls was 25 years (range 20–30) whereas that of patients with OA was 55 years (range 49–65). Patients with OA were taking nonsteroidal antiinflammatory drugs or analgesics on a need basis.

Serum and SF IL-17 concentration. Serum IL-17 was detectable in 3 of the 10 patients with JIA as compared to none in the healthy control group. The median SF IL-17 levels were higher in patients with JIA [28 pg/ml (range 0–200)] compared to OA [0 pg/ml (range 0–84); $p < 0.001$]. The SF levels were also higher in patients with JIA [28 pg/ml (range 0–200)] compared to RA [0 pg/ml (range 0–136); $p < 0.05$] (Figure 1A).

The levels were comparable between patients with poly-JIA [28 pg/ml (range 0–200)] and the ERA group [28 pg/ml (range 0–160)] (Figure 1B). Paired serum and SF analysis from 10 patients with JIA showed higher levels in SF [31 pg/ml (range 16–200)] compared to serum [0 pg/ml (range 0–11); $p < 0.005$] (Figure 1C).

Correlation of SF IL-17 levels with other cytokines and measures of disease activity. In the ERA group, the median IL-6, IL-1ß, and TNF-α levels were 1125.8 pg/ml (range 4–2187.6), 0 pg/ml (range 0–212), and 90 pg/ml (range 0–2000), respectively. SF IL-17 levels did not correlate with levels of these inflammatory cytokines. In the ERA group, SF IL-17 correlated with number of swollen joints ($r = 0.35$; $p < 0.05$), number of joints with limited mobility ($r = 0.55$; $p < 0.001$), and

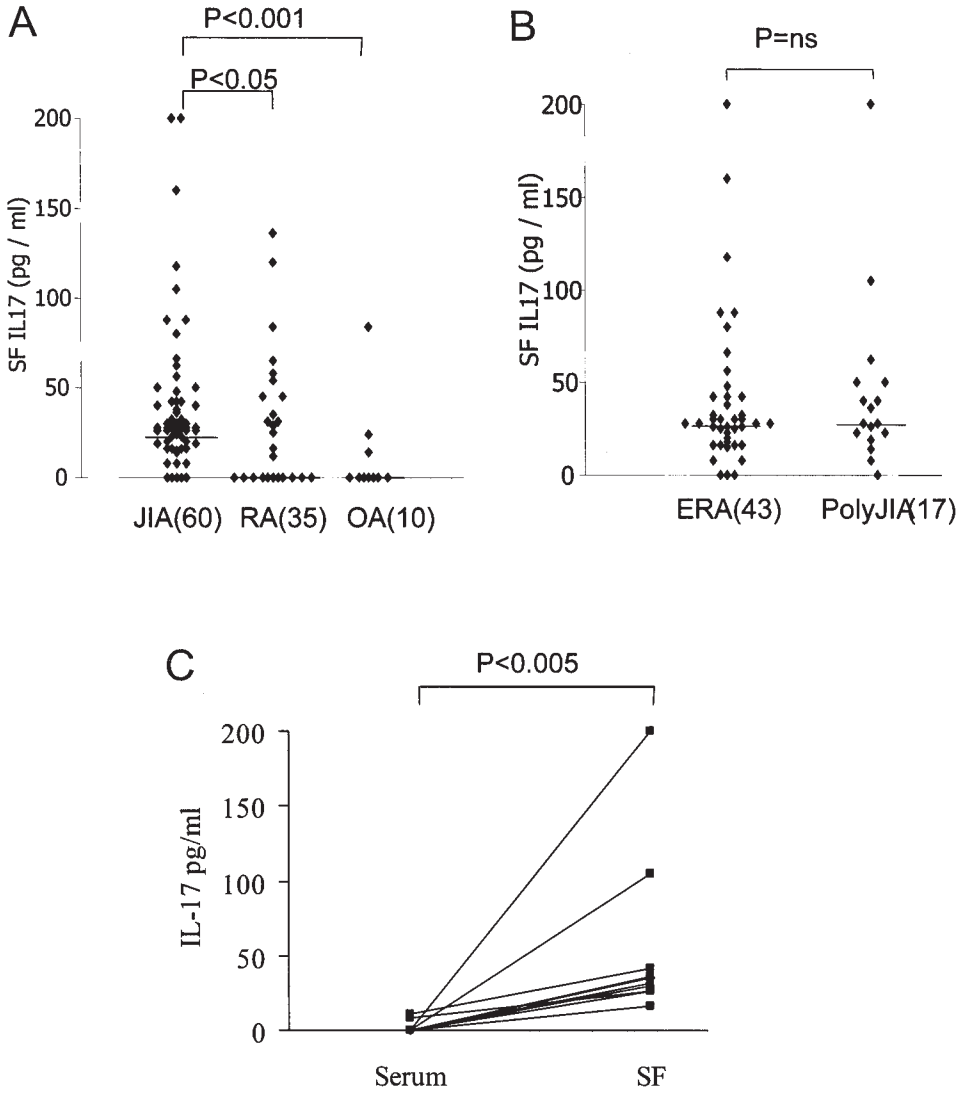

Figure 1. Synovial fluid (SF) levels of IL-17 in (A) juvenile idiopathic arthritis (JIA), rheumatoid arthritis (RA), and osteoarthritis (OA). (B) Comparison between enthesitis-related arthritis (ERA) and polyarticular JIA. (C) Paired serum and SF levels in 10 patients with JIA. Values in the parentheses show number of patients. Inter-group comparisons by Mann-Whitney U-test.

number of tender joints ($r = 0.46$; $p < 0.01$); however, no correlation was seen with erythrocyte sedimentation rate (ESR).

In poly-JIA, the median IL-6, IL-1ß, and TNF-α levels were 833 pg/ml (range 17.4–2119), 48.5 pg/ml (range 0–164), and 90 pg/ml (range 0–200), respectively. SF IL-17 levels did not correlate with levels of these inflammatory cytokines, or with number of swollen joints, number of tender joints, or ESR. SF IL-17 levels correlated with number of joints with limited mobility ($r = 0.54$; $p < 0.05$).

In vitro stimulation of FLS with IL-17. Induction of IL-6 and IL-8 by stimulation of FLS with IL-17 and TNF-α. Four different ERA synovial fibroblast cultures and 2 different RA FLS cultures were stimulated with TNF-α and IL-17 or were left untreated. TNF-α and IL-17 increased the production of IL-6 over baseline by 8.2-fold and 8.7-fold, respectively (Figure 2A) and IL-8 by 27-fold and 11.2-fold, respectively (Figure 2B). FLS derived from JIA had similar stimulation as those derived from RA.

Production of MMP-3 and MMP-1 by FLS. Both TNF-α and IL-17 significantly increased the production of MMP-3 over baseline by 6-fold and 2.5-fold, respectively (Figure 2C). Both TNF-α and IL-17 also significantly increased the production of MMP-1 over baseline by 2-fold and 2.65-fold, respectively (Figure 2D).

Effect on TIMP production by synovial fibroblasts. There was no effect on TIMP production after stimulation by TNF-α and IL-17 compared to basal (Figure 2E).

DISCUSSION

We have described elevated IL-17 levels in patients with ERA

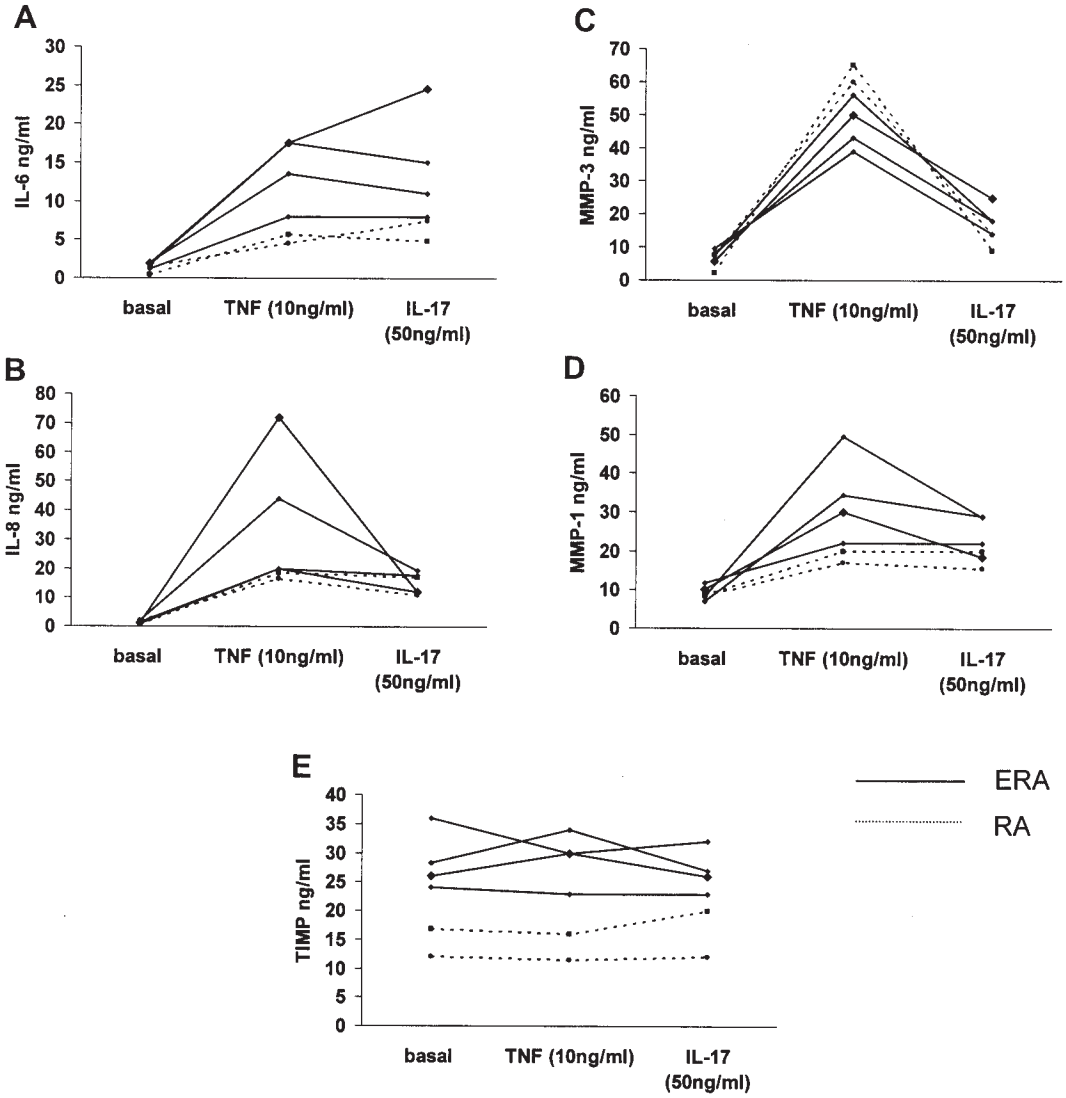

Figure 2. Production of IL-6 (A), IL-8 (B), MMP-3 (C) MMP-1 (D), and TIMP (E) by ERA and RA fibroblast-like synoviocytes (FLS) in the presence of TNF-α and IL-17. Four different ERA FLS cultures and 2 different RA FLS cultures were stimulated with TNF-α and IL-17 or were left untreated. Each line represents data from individual patients' FLS.

and poly-JIA compared to OA. The levels were higher in SF compared to serum. SF IL-17 levels correlated with measures of disease activity. Further, IL-17 stimulation of FLS derived from ERA led to production of proinflammatory cytokines and MMP.

We found elevated IL-17 levels in SF of patients with JIA. In RA, 2 studies have shown elevated levels of serum and SF in small numbers of patients[10,18]. IL-17 is produced by Th17 cells, which are present in RA synovium. IL-17 stimulates the monocytes to produce proinflammatory cytokines such as IL-1ß, TNF-α, and IL-6, thus amplifying the inflammatory cascade[11]. Elevated levels of IL-1ß, TNF-α, and IL-6 are reported in patients with JIA[19]. Lack of correlation between IL-17 levels and other proinflammatory cytokines in our study suggests that these are regulated by multiple stimuli. Elevated levels of IL-17 in JIA SF suggest a role for this cytokine in synovitis and joint destruction in JIA as has been reported in RA. This is also supported by correlation of SF IL-17 levels with measures of disease activity in our study.

Higher SF IL-17 levels as compared to serum levels suggest local production of this cytokine. Absence of any difference in serum levels between patients and controls further supports local production. In contrast, a recently published study, which found no difference in plasma levels between patients with JIA and healthy controls, found IL-17 levels to be higher in plasma as compared to SF in oligoarticular and polyarticular JIA, whereas the reverse was found in systemic onset JIA[14].

IL-17 induced production of IL-6, IL-8, MMP-3, and MMP-1 from FLS derived from patients with ERA, but no effect was seen on TIMP. It has been reported that Th17 cells have a crucial role in autoimmune inflammation[7]. IL-17 has been shown to increase the spontaneous production of MMP-1, and the presence of anti-IL-17-blocking antibody reduced MMP-1 production and collagenase activity by RA synovium[20]. Addition of IL-17 enhanced IL-6 production and collagen destruction and inhibited collagen synthesis by RA synovium explants[11]. IL-17 alone released proteoglycan and type II collagen from bovine nasal cartilage explants, and in IL-17-treated chondrocytes, mRNA expression for MMP-1, MMP-3, and MMP-13 was detected[21]. Data from animal models also suggest a role of IL-17 in cartilage degradation. Suppression of induction of CIA was found in IL-17-deficient mice[12]. Treatment with a neutralizing anti-IL-17 antibody after the onset of CIA significantly reduced the severity of arthritis. Suppression of joint inflammation, prevention of cartilage and bone destruction, and significant reduction of IL-6 levels were found after addition of anti-IL-17 antibody[13]. IL-17 receptor deficiency results in impaired synovial expression of IL-1 and MMP-3, MMP-9, and MMP-13 and prevents cartilage destruction during chronic reactivated streptococcal cell wall-induced arthritis[22].

Since IL-17 induced proinflammatory cytokine production it is possible that the effect on MMP production is mediated through these cytokines rather than directly by IL-17. Blocking experiments with anti-IL-6 and anti-IL-8 antibodies would have helped define this.

Patients with ERA and poly-JIA have elevated SF IL-17 levels, and its correlation with measures of disease activity suggests that IL-17 may play an important role in pathogenesis of synovitis in patients with ERA and poly-JIA. In addition, IL-17 specifically induces MMP expression in ERA synovial fibroblasts, without inducing TIMP, which may suggest a role of this cytokine in cartilage destruction.

REFERENCES

1. Sullivan KE. Inflammation in juvenile idiopathic arthritis. Pediatr Clin North Am 2005;52:335-57.
2. Gottorno M, Gerloni V, Morando A, et al. Synovial membrane expression of matrix metalloproteinases and tissue inhibitor-1 in juvenile idiopathic arthritides. J Rheumatol 2002;29:1774-9.
3. Gottorno M, Vignola S, Falcini F, et al. Serum and synovial fluid concentrations of matrix metalloproteinases 3 and its tissue inhibitor 1 in juvenile idiopathic arthritides. J Rheumatol 2002;29:826-31.
4. Peake NJ, Cawston TE, Rowan AD, et al. Levels of matrix metalloproteinase MMP1 in paired sera and synovial fluids of juvenile idiopathic arthritis patients: relationship to inflammatory activity, MMP3 and tissue inhibitor of metalloproteinases-1 in a longitudinal study. Rheumatology Oxford 2005;44:1383-9.
5. MacNaul KL, Chartain N, Larks M, et al. Discordinate expression of stromelysin, collagenase, and tissue inhibitor of metalloproteinases-1 in rheumatoid human synovial fibroblasts. J Biol Chem 1990;265:17238-45.
6. Weaver CT, Harrington LE, Mangan PR, Gavrieli M, Murphy KM. Th17: an effector of CD4 T cell lineage with regulatory T cell ties. Immunity 2006;24:677-88.
7. Fossiez F, Djossou O, Chomarat P, et al. T cell interleukin-17 induces stromal cells to produce proinflammatory and hematopoietic cytokines. J Exp Med 1996;183:2593-603.
8. Miossec P. Interleukin-17 in rheumatoid arthritis: if T cells were to contribute to inflammation and destruction through synergy. Arthritis Rheum 2003;48:594-601.
9. Sato K, Suematsu A, Okamoto K, et al. Th17 functions as an osteoclastogenic helper T cells subset that links T cell activation and bone destruction. J Exp Med 2006;203:2673-82.
10. Kotake S, Udagawa N, Takahashi N, et al. IL-17 in synovial fluid from patients with rheumatoid arthritis is a potent stimulator of osteoclastogenesis. J Clin Invest 1999;103:1345-52.
11. Chabaud M, Lubberts E, Joosten L, van den Berg W, Miossec P. IL-17 derived from juxta-articular bone and synovium contributes to joint degradation in rheumatoid arthritis. Arthritis Res 2001;3:168-77.
12. Nakae S, Nambu A, Sudo K, Iwakura Y. Suppression of immune induction of collagen-induced arthritis in IL-17-deficient mice. J Immunol 2003;171:6173–7.
13. Lubberts E, Konders MI, Oppers-Walgreen B, et al. Treatment with a neutalizing anti-murine IL-17 antibody after the onset of collagen-induced arthritis reduces joint inflammation, cartilage destruction and bone erosion. Arthritis Rheum 2004;50:650-9.
14. de Jager W, Hoppenrejis EPAH, Wulffraat NM, Wedderburn LR, Kuis W, Prakken BJ. Blood and synovial fluid cytokine signatures in patients with juvenile idiopathic arthritis: a cross sectional study. Ann Rheum Dis 2007;66:589-98.
15. Aggarwal A, Agarwal V, Danda D, Misra R. Outcome in juvenile rheumatoid arthritis in India. Indian Pediatrics 2004;41:180-4.
16. Petty RE, Southwood TR, Baum J, et al. Revision of the proposed classification criteria for juvenile idiopathic arthritis; Durban, 1997. J Rheumatol 1998;25:1991-4.
17. Stebulis JA, Rossetti RA, Atez FJ, Zurier RB. Fibroblast like synovial cells derived from synovial fluid. J Rheumatol 2005;32:301-6.
18. Ziolkowska M, Koc A, Luszczykiewicz G, et al. High levels of IL-17 in rheumatoid arthritis patients: IL-15 triggers in vitro IL-17 production via cyclosporin A-sensitive mechanism. J Immunol 2000;164:2832-8.
19. Saxena N, Aggarwal A, Misra R. Elevated concentrations of monocyte derived cytokines in synovial fluid of children with enthesitis related arthritis and polyarticular types of juvenile idiopathic arthritis. J Rheumatol 2005;32:1349-53.
20. Chabaud M, Garnero P, Dayer JM, Guerne PA, Fossiez F, Miossec P. Contribution of interleukin 17 to synovium matrix destruction in rheumatoid arthritis. Cytokine 2000;12:1092-9.
21. Koshy PJ, Henderson N, Logan C, Life PF, Cawston TE, Rowan AD. IL-17 induces cartilage collagen breakdown: novel synergistic effects in combination with proinflammatory cytokines. Ann Rheum Dis 2002;61:704-13.
22. Konders MI, Kolls JK, Oppers-Walgreen B, et al. IL-17 receptor deficiency results in impaired synovial expression of IL1 and matrix metalloproteinases 3, 9, and 13 and prevents cartilage destruction during chronic reactivated Streptococcal cell wall induced arthritis. Arthritis Rheum 2005;52:3239-47.