Mod Rheumatol (2008) 18:15–22
DOI 10.1007/s10165-007-0015-y

ORIGINAL ARTICLE

Interleukin-17 gene expression in patients with rheumatoid arthritis

Mika Kohno · Akito Tsutsumi · Hiroto Matsui · Makoto Sugihara · Takeshi Suzuki · Mizuko Mamura · Daisuke Goto · Isao Matsumoto · Satoshi Ito · Toru Suguro · Takayuki Sumida

Received: 14 August 2007 / Accepted: 28 September 2007 / Published online: 20 December 2007

Abstract Interleukin-17 is a proinflammatory cytokine. Recent animal studies have shown that IL-17 plays a role in the initiation and progression of arthritis. However, whether IL-17 has a prominent role in human rheumatoid arthritis (RA) or not remains unclear. Here we investigated the role of IL-17 in patients with RA. cDNA was prepared from knee joint synovial tissues of RA ($n = 11$) and osteoarthritic (OA, $n = 10$) patients and PBMC of RA ($n = 52$) and healthy subjects ($n = 34$). IL-17 gene expression level was measured by real-time PCR, and was compared with various clinical parameters. IL-17 gene expression in synovial tissues of RA was similar to that in OA. IL-17 gene expression level in PBMC of RA patients was significantly higher than in the control. The response (changes in DAS) to two-week treatment with anti-TNF-α blockers (infliximab or etanercept) did not correlate with changes in IL-17 gene expression levels. The IL-17/TNF-α gene expression ratio at baseline (before treatment) tended to be lower in responders to the treatment. Expression of IL-17 gene in PBMC may be associated with the inflammatory process of RA. IL-17/TNF-α expression ratio is a potentially suitable marker of response to anti-TNF-α therapy.

M. Kohno · A. Tsutsumi · H. Matsui · M. Sugihara · T. Suzuki · M. Mamura · D. Goto · I. Matsumoto · S. Ito · T. Sumida (✉)
Division of Clinical Immunology, Major of Advanced Biomedical Applications, Graduate School Comprehensive Human Science, University of Tsukuba,
1-1-1 Tennnodai, Tsukuba 305-8575, Japan
e-mail: tsumida@md.tsukuba.ac.jp

T. Suguro
Department of Orthopaedic Surgery, School of Medicine, Toho University, Tokyo, Japan

Keywords Etanercept · Infliximab · Interleukin-17 (IL-17) · Rheumatoid arthritis · Tumor necrosis factor-α (TNF-α)

Introduction

Rheumatoid arthritis (RA) is characterized by chronic synovial inflammation, cartilage degradation and bone erosion in multiple joints, which ultimately lead to joint destruction and disability. Inflammatory cytokines such as tumor necrosis factor α (TNF-α), interleukin-1 (IL-1) and IL-6 are involved in the pathogenesis of RA [1–3], and the beneficial effects of antagonists to these cytokines have been reported [4–6]. However, not all patients respond to these agents, suggesting that other pro-inflammatory cytokines, e.g., IL-17, may be important in the pathogenesis of RA.

IL-17 is a proinflammatory cytokine produced by activated and memory $CD4^+$ $CD45RO^+$ T cells [7–8], and is a potent inducer of other cytokines, such as TNF-α, IL-1β, IL-6, IL-8, and granulocyte colony stimulating factor (G-CSF) in a variety of epithelial, endothelial, and fibroblastic cell types [9]. In experimental arthritis, IL-17 was found to be important in both the early initiation phase and late progression phase, especially in arthritis models driven mainly by T cells, such as the IL-1 $Ra^{-/-}$ mouse model and streptococcal cell wall-induced arthritis [10–15].

Several studies have suggested that IL-17 plays a role in the pathogenesis of RA. Chabaud et al. [16] reported that the proportion of synovial membrane cultures that produced IL-17 was higher in those from RA patients than those from OA patients or healthy controls. In addition, previous studies indicated the presence of elevated IL-17 levels in the synovial fluid of patients with sRA [17, 18].

Although these observations imply that IL-17 is involved in the pathogenesis of human RA, there is no solid information on whether IL-17 actually plays a role in RA pathogenesis.

With regard to TNF-α, previous studies reported that IL-17 contributes to the arthritic process not only by inducing the production of TNF-α, but also acting in synergy with or independently of TNF-α [8, 19–23]. Although it is unknown whether these observations are relevant in human RA, we hypothesized that IL-17 gene expression level in PBMC might be enhanced independent of that of TNF-α in some patients with RA, and that these patients may comprise a subpopulation of RA refractory to anti-TNF-α therapy.

The aims of the present study were to clarify the role of IL-17 in disease activity and progression of human RA and to explore the possibility of IL-17 as a target for the treatment for RA. For this purpose, we measured the gene expression of IL-17 in peripheral blood mononuclear cells (PBMC) and synovial tissues from RA patients. We correlated the findings of PBMC samples with various clinical parameters, TNF-α gene expression and the efficacy of infliximab or etanercept.

Methods

Patients

Peripheral blood was taken from 52 patients who fulfilled the 1987 American Rheumatism Association criteria for the classification of RA [24]. The patient group comprised 19 males and 33 females (age, 25–84 years, mean ± SD: 54.0 ± 13.0 years). The fifty-two RA patients included 25 infliximab- and 11 etanercept-treated patients who attended our unit between 29 September 2003 and 22 August 2006, and, as a control group, 16 RA patients who visited our unit between October 11 and October 25 2006. Clinical data such as white blood cell count (WBC), erythrocyte sediment rate (ESR), c-reactive protein (CRP) and matrix metalloproteinase 3 (MMP-3) was obtained at the time of blood sampling. We also included a group of control healthy donors (13 men and 21 women, age 30.0 ± 5.0 years). Synovial tissues were obtained at the time of total knee replacement performed from April 2001 to June 2002 on 11 RA patients (one male and ten females, age 56.8 ± 8.7 years) and ten osteoarthritis (OA) patients (ten females, age 73.4 ± 2.7 years). No patient underwent both blood and synovial tissue sampling. Patient demographics are listed in Table 1. Written informed consent was obtained from all subjects, and the study was approved by the appropriate ethics committee.

Treatment with infliximab or etanercept and assessment of efficacy

Twenty-five RA patients were treated with 3 mg/kg of infliximab at weeks 0, 2, 6, and 14, and every eight weeks thereafter. Another RA group consisting of 11 patients were treated with etanercept (25 mg injection twice weekly). Drug efficacy was evaluated by comparing the differences in European League Against Rheumatism improvement criteria [Disease Activity Score (DAS)] at week 0 (before treatment) and at 2 weeks.

Synovial and blood samples for cDNA synthesis

Part of each synovial tissue was cut into small pieces and rinsed with phosphate-buffered saline (PBS). Complementary DNA (cDNA) was prepared from synovial tissues

Table 1 Demographics of the subjects included in the study

PBMC	Mean ± SD	Range	Synovium	Mean ± SD	Range
RA patients			RA patients		
Age (years)	54.0 ± 13.0	(25–84)	Age (years)	56.8 ± 8.7	(37–70)
Males	19		Males	1	
Females	33		Females	10	
C-reactive protein (mg/dl)	2.15 ± 1.63	(0.25–8.95)	C-reactive protein (mg/dl)	1.65 ± 2.64	(0.07–7.17)
ESR (mm/h)	48.5 ± 21.1	(12–104)	ESR (mm/h)	33.4 ± 25.1	(10–95)
Rheumatoid factor (IU/ml)	245 ± 231	(5–1790)	Rheumatoid factor (IU/ml)	161 ± 199	(4–715)
Healthy controls			OA patients		
Age (years)	30.0 ± 5.0	(22–50)	Age (years)	73.4 ± 2.7	(69–78)
Males	13		Males	0	
Females	21		Females	10	

ESR erythrocyte sedimentation rate

using Revertaid first strand cDNA synthesis kit (Fermentas, Hanover, MD, USA), following the instructions provided by the manufacturer.

Peripheral blood mononuclear cells (PBMC) were isolated from heparinized peripheral blood obtained from normal subjects and RA patients using Ficoll-Paque PLUS (Amersham Biosciences, Uppsala, Sweden) following the protocol recommended by the manufacturer. Cells were spun down to pellets and total RNA was extracted from the cell pellets using Isogen (Nippongene, Tokyo, Japan). The cDNAs were synthesized using a Revertaid first strand cDNA synthesis kit (Fermentas), following the instructions provided by the manufacturer. These cDNA samples underwent gene expression analyses.

Quantification of gene expression by real-time polymerase chain reaction

The cDNA samples were amplified with specific primers and fluorescence-labeled probes for the target genes. Specific primers and probes for IL-17, IL-6. TNF-α and glyceraldehyde-2-phosphate dehydrogenase (GAPDH) were purchased from Applied Biosystems Japan (Tokyo). The amplified product genes were monitored on an ABI 7700 sequence detector (Applied Biosystems Japan). The qPCR master mix was also purchased from Applied Biosystems Japan. The final concentrations of the primers were 200 nM for each of the 5′ and 3′ primers, and the final probe concentration was 100 nM. The thermal cycler conditions used were 50 °C for 2 min, 95 °C for 10 min, then 50 cycles of 95 °C for 15 s and 60 °C for 1 min. Serial dilutions of a standard sample were included in every assay, and standard curves for the genes of interest and GAPDH genes were generated. All measurements were performed in triplicate. The level of gene expression was calculated from the standard curve, and expressed relative to GAPDH gene expression.

Statistical analysis

All data were expressed as mean ± standard deviation (SD). The Mann–Whitney U test was used to compare the expression levels of genes between RA patients and healthy controls. Spearman's correlation coefficient by rank test was calculated to assess the correlations between the expression levels of IL-17 and TNF-α genes, as was the correlation between IL-17/TNF-α gene expression ratio and changes in DAS after two weeks of therapy with etanercept or infliximab relative to baseline (before treatment, i.e., DAS 0–2 weeks). Paired Wilcoxon's rank test was used to compare the gene expression levels between week 0 and week 2. A P value of less than 0.05 was considered significant.

Results

Expression levels of IL-17 genes in synovial tissues

We anticipated that the expression of IL-17 would be enhanced in RA patients at the site of inflammation, namely the synovial tissue. However, the expression of IL-17 gene in synovial tissues of RA patients, as measured by real-time PCR, was not significantly different from that of the OA patients (Fig. 1).

Expression levels of IL-17 genes in PBMC

We next asked whether the expression level of IL-17 gene is upregulated in PBMC of RA patients. Expression of the IL-17 gene in PBMC from RA patients was significantly higher than that of the control (RA: 0.0437 ± 0.1112, control: 0.0134 ± 0.0033, $P = 0.011$, Fig. 2).

Relationship between IL-17 and IL-6 or TNF-α gene expression in RA patients

We wished to determine whether the expression of IL-17 gene in patients with RA is significantly associated with

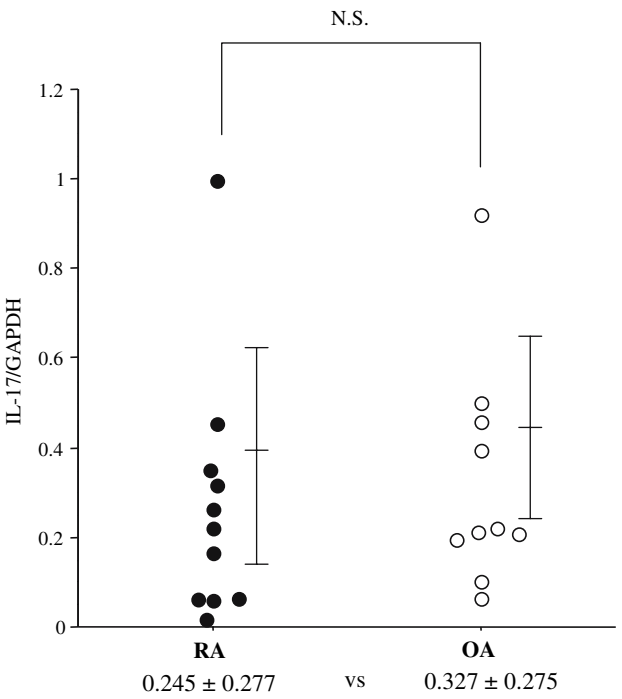

Fig. 1 Expression levels of IL-17 gene in the synovium tissues of patients with RA (*closed circles*, $n = 11$) and osteoarthritis (OA, *open circles*, $n = 10$). Data are mean ± SD. The P value was calculated by the Mann–Whitney U test

expressions of other inflammatory cytokines such as TNF-α and IL-6. No significant relationship between expression of the IL-17 gene and that of IL-6 or TNF-α was observed (Fig. 3a, b). In addition, there was no significant relationship between the expression level of IL-6 and that of TNF-α (Fig. 3c). Furthermore, the expression levels of IL-17 in PBMC of RA patients did not correlate significantly with WBC count, serum CRP, ESR or serum MMP-3 (data not shown).

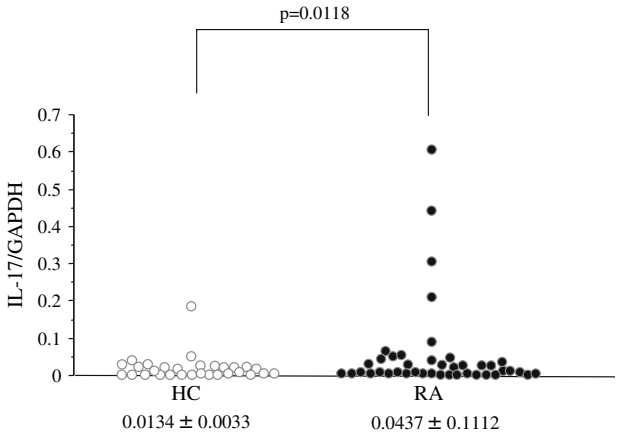

Fig. 2 Expression levels of IL-17 gene in peripheral blood mononuclear cells from patients with RA (*closed circles*, $n = 52$) and from healthy controls (HC, *open circles*, $n = 34$). Data are mean ± SD. The *P* values were calculated by the Mann–Whitney U test

Effects of infliximab and etanercept therapy on IL-17 gene expression in PBMC

We compared the gene expression levels of IL-17 in PBMC samples at baseline, and two weeks after the first infusion or injection of biologics that target TNF-α. In patients who received infliximab, no significant differences were observed between baseline and week 2 samples (week 0: 0.089 ± 0.157, week 2: 0.044 ± 0.056, Fig. 4a). On the other hand, etanercept significantly increased IL-17 gene expression at two weeks after initiation of therapy (week 0: 0.041 ± 0.132, week 2: 0.134 ± 0.274, $P = 0.028$, Fig. 4 b). There were no relationships between changes in IL-17 gene expression levels and efficacy (changes in DAS) of infliximab and etanercept.

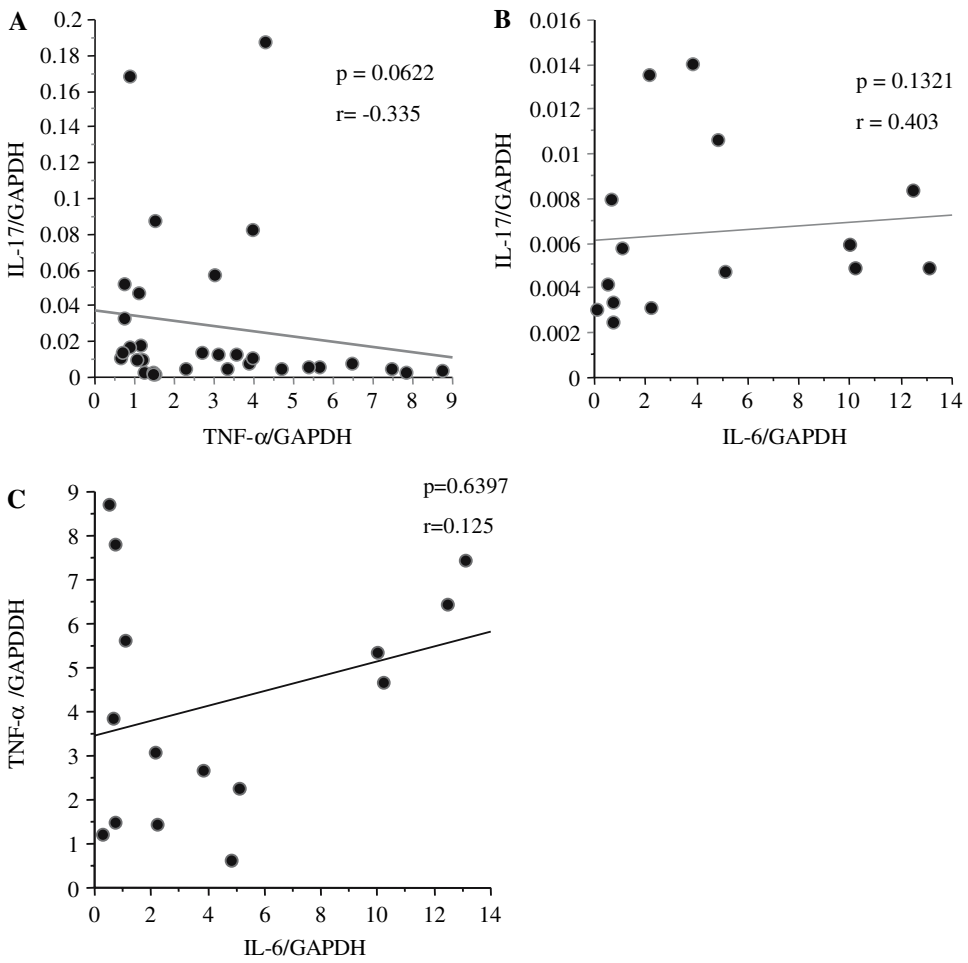

Fig. 3 Relationships between expression levels of IL-17, TNF-α and IL-6 genes in peripheral blood mononuclear cells from rheumatoid arthritis (RA) patients. **a** Relationship between expression levels of IL-17 and TNF-α in PBMC from RA ($n = 31$). **b** Relationship between expression levels of IL-17 and IL-6 in PBMC from RA ($n = 15$). **c** Relationship between expression levels of TNF-α and IL-6 in PBMC from RA ($n = 15$) r = correlation coefficient. The *P* values were calculated by Spearman's correlation coefficient by rank test

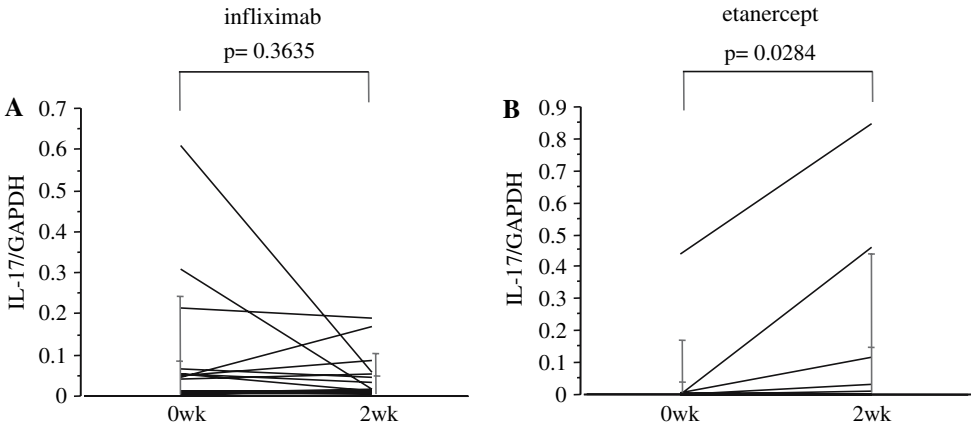

Fig. 4 IL-17 gene expression levels in peripheral blood mononuclear cells at baseline and two weeks after the first infusion of infliximab (n = 17) (**a**), and after the first injection of etanercept (n = 11) (**b**). Data are mean ± SD. The P values were calculated by Wilcoxon's rank sum test

Relationships between IL-17 and TNF-α gene expression and efficacy of anti-TNF-α therapy

We next examined whether patients with a high expression level of IL-17 gene relative to that of the TNF-α gene were more refractory to anti-TNF-α therapy. For this purpose, we calculated the IL-17/TNF-α gene expression ratio in PBMC of RA patients prior to anti-TNF-α therapy and correlated it with changes in DAS from pre- to two-week post-therapy (DAS0-2 week). The results showed that the IL-17/TNF-α gene expression ratio prior to treatment tended to be lower in patients who responded to anti-TNFα therapy, although the relationship was not statistically significant (Fig. 5).

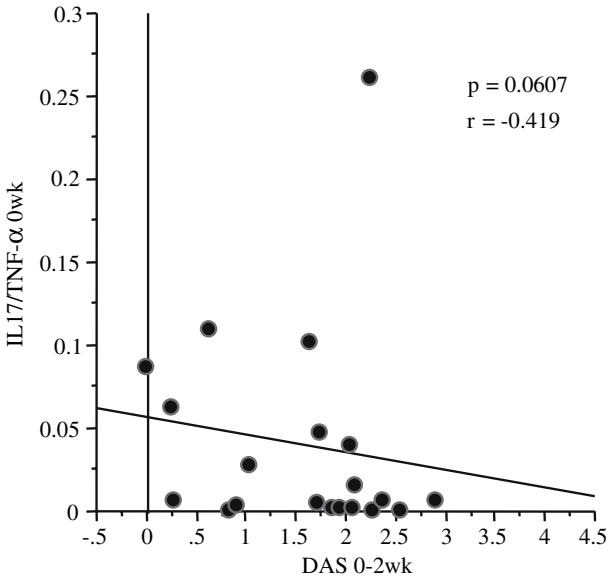

Fig. 5 Relationship between IL-17/TNF-α ratio in peripheral blood mononuclear cells and response to anti-TNF-α therapy. Number of patients = 20, r = correlation coefficient. The P values were calculated by Spearman's correlation coefficient by the rank test

Discussion

The active involvement of IL-17 in both the initiation stage and the progression stage of murine experimental arthritis has been demonstrated and IL-17 is considered a key cytokine in the pathogenesis of arthritis in such experimental models [10–13]. A few studies suggest that IL-17 may have a role in the pathogenesis of human RA as well [16–18].

Based on the above background, we measured IL-17 gene expression in synovial tissues and PBMC of RA patients, and compared them with those in control samples. We first compared the expression of IL-17 gene in synovial tissues from RA patients with that in OA patients. Unexpectedly, the IL-17 gene expression level in synovial tissues of OA patients was comparable to that in RA patients. This result is in contrast to that described by Chabaud et al. [16], where expression of IL-17 gene was higher in RA synovium than in OA synovium. Differences in patient demographics and sample size may partly account for this discrepancy. While OA is not generally considered an inflammatory disorder, it is reported that IL-17 upregulates the release of IL-8 and GRO-α in synovial fibroblasts and chondrocytes isolated from patients from OA, suggesting that IL-17 does play a role in the joint destruction process of OA [25]. In this study, we were unable to obtain synovial tissues from healthy individuals, which would have been a better control for this study. In contrast to our study, Kotake et al. [17] reported the presence of high IL-17 protein levels in synovial fluids of patients with RA, but not those with OA. The reason for this discrepancy is unclear, but it is possible that differences in post-transcription regulation of IL-17 production between RA and OA partly contributes to the difference in protein levels in the synovial fluid.

We next examined whether IL-17 gene expression in PBMC is elevated in patients with RA. As expected, the expression of IL-17 gene was significantly higher in PBMC of RA patients than in those of healthy controls. This result

implies that IL-17 does have a role in the inflammatory process of RA, at least in some patients. We did not measure expression of the IL-17 gene in PBMC from OA patients. Comparison of IL-17 gene expression in synovial tissues and PBMC taken simultaneously in both RA patients and OA patients could be informative. Expression of the IL-17 gene in PMBC of RA patients differed greatly among individuals, suggesting that such expression is important in some patients at some points during the course of the disease. Future studies with serial samples from RA patients would be informative. In this regard, IL-17 protein levels were undetectable in serum samples of 15 RA and 15 healthy controls (<15.6 pg/ml) by enzyme immunoassay (data not shown). Thus, we were unable to determine whether IL-17 gene expression levels in PBMC reflect the levels of serum IL-17 protein.

Interleukin-17 is known to enhance the pro-inflammatory effects of TNF-α in vitro and to act in synergy with TNF-α in the progression of arthritis in experimental arthritis mouse models [8, 19, 20, 21]. IL-17 is reported to depend strongly on TNF-α in induction of arthritis under naïve conditions in another experimental arthritis model [15]. However, it was recently reported that IL-17 could induce arthritis in the absence of TNFα in an experimental arthritis model [22]. Therefore, we considered it possible that (1) the expression of IL-17 gene in PBMC may be related to that of TNF-α, or (2) some RA patients show enhanced IL-17 gene expression level in PBMC regardless of the TNFα gene expression level, and comprise a subpopulation of anti-TNF-α refractory patients. We found a tendency towards a negative relationship between the expression of IL-17 gene and that of TNF-α gene in PBMC of RA patients, although the relationship was not significant. In addition, we found no significant relationship between IL-17 gene expression in PBMC and various parameters of inflammation, including ESR and serum CRP. These results imply that, in RA, which is a multifactorial and heterogeneous disease, gene expressions of TNF-α and IL-17 in PBMC are not directly associated with each other, and the expression of IL-17 in PBMC does not have an overwhelming influence on the inflammatory status.

Interleukin-6 is also a pro-inflammatory cytokine known to play an important role in the pathogenesis of RA [26, 27], and also to regulate the differentiation of Th17 T cells. Previous studies indicated that IL-6$^{-/-}$CD4+ T cells from draining lymph nodes produced less IL-17 than cells from wild-type mice, and that IL-6-deficient SKG mice were completely devoid of IL-17^{+}CD4+ T cells [28–30]. Conversely, IL-17 induced secretion of IL-6 from cultured fibroblasts [7, 8]. Based on this background, we compared the expression of IL-17 and IL-6 genes in PBMC of RA patients. In contrast to the relationship between IL-17 gene expression and TNF-α gene expression, a tendency towards a positive relationship was observed, albeit statistically insignificant.

It has been reported that IL-23 is important in the survival and expansion of IL-17- and IL-6-producing Th17 T cells and the development of collagen-induced arthritis in mice [30–33]. A recent study reported that self-reactive T cells produced by genetic alteration of thymic T cell selection spontaneously differentiate into Th17 T cells, and that these T cells stimulate antigen presenting cells (APC) to secrete IL-6 [29]. APC-derived IL-6 and T-cell derived IL-6 drive naïve T cells to differentiate into arthritogenic Th17 T cells [34]. Our observation may reflect the fact that Th17 T cells have an important role in the pathogenesis of human RA.

The efficacy of anti-TNF-α therapy in RA is well established [35]. However, non-responders hardly show any improvement in symptoms even after continuous injection or infusion of TNF-α antagonists. These observations imply that some cytokines other than TNF-α can act independently of TNF-α in the pathogenesis of RA. If IL-17 is one of these cytokines, IL-17 may become an appropriate target for treatment of RA patients refractory to TNF-α antagonists. To gain an insight into this question, we first addressed how TNF-α blockage affected the expression of IL-17 gene in PBMC of patients with RA by comparing IL-17 gene expression before and two weeks after the first infliximab or etanercept injection. Infliximab did not significantly affect IL-17 expression but etanercept significantly increased the expression in PBMC after two weeks of therapy. The reason for this difference is not clear at present. However, etanercept is known to be a decoy receptor and inhibitor of the action of soluble TNF-α, while infliximab is an antibody against TNF-α and is reported to induce negative signals through membrane TNF-α [36]. This may partly account for the observed differences in the action of these two agents. At present, we could not find any significant relationship between the efficacy of TNF-α blockade therapy and fluctuation of IL-17 gene expression in PBMC. A study in a larger number of patients is warranted to examine this issue.

We also calculated the IL-17/TNF-α ratio in PBMC of RA patients prior to initiation of therapy using TNF-α blocking agents, and examined the changes in the ratio after such treatment. While there was no relationship between DAS 0–2 week and IL-17 or TNF-α gene expression, the IL-17/TNF-α ratio tended to be lower in responders. These results suggest that RA patients with low IL-17 gene expression and high TNF-α gene expression in PBMC before treatment are more likely to respond to anti-TNF-α therapy. Currently there are no tools available to distinguish between responders and non-responders before TNF-α blockade therapy. IL-17/TNF-α expression ratio in

PBMC may be a suitable predictor of the response to treatment with TNF-α blockers.

In conclusion, we have demonstrated that IL-17 gene expression in PBMC of RA patients is higher than those in controls. We speculate that IL-17 might play an important role in the pathogenesis of RA, and that IL-17/TNF-α gene expression ratio in PBMC prior to infliximab or etanercept therapy may predict the response to treatment. Further studies are necessary to clarify the molecular basis of IL-17 action in the pathogenesis of RA and whether IL-17 is a suitable target for the treatment of RA.

References

1. Arner EC, Pratta MA. Independent effects of interleukin-1 on proteoglycan breakdown, proteoglycan synthesis, and prostaglandin E_2 release from cartilage in organ culture. Arthritis Rheum 1989;32:288–97.
2. Arend WP, Dayer J-M. Inhibition of the production and effects of interleukin-1 and tumor necrosis factor α in rheumatoid arthritis. Arthritis Rheum 1995;38:151–60.
3. Van de Loo FAJ, Joosten LAB, van Lent PLEM, Arntz OJ, van den Berg WB. Role of interleukin-1, tumor necrosis factor α, and interleukin-6 in cartilage proteoglycan metabolism and destruction: effect of in situ blocking in murine antigen- and zymosan-induced arthritis. Arthritis Rheum 1995;38:164–72.
4. Shanahan JC, St Clair W. Tumor necrosis factor alpha blockade: a novel therapy for rheumatic disease. Clin Immunol 2002;103:231–42.
5. Nishimoto N, Yoshizaki K, Miyasaka N, Yamamoto K, Kawai S, Takeuchi T, et al. Treatment of rheumatoid arthritis with humanized anti-interleukin-6 receptor antibody: a multicenter, double-blind, placebo-controlled trial. Arthritis Rheum 2004;50:1761–9.
6. Dayer JM, Feige U, Edwards CK 3rd, Burger D. Anti-interleukin-1 therapy in rheumatic diseases. Curr Opin Rheumatol 2001;13:170–6.
7. Yao Z, Painter SL, Fanslow WC, Ulrich D, Macduff BM, Spriggs MK, et al. Human IL-17: a novel cytokine derived from T cells. J Immunol 1995;155:5483–6.
8. Fossiez F, Djossou O, Chomarat P, Flores-Romo L, Ait-Yahia S, Maat C, et al. T cell interleukin-17 induces stromal cells to produce proinflammatory and hematopoietic cytokines. J Exp Med 1996;183:2593–603.
9. Aggarwal S, Gurney AL. IL-17: prototype member of an emerging cytokine family. J Leukoc Biol 2002;71:1.
10. 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.
11. Nakae S, Saijo S, Horai R, Sudo K, Mori S, Iwakura Y. IL-17 production from activated T cells is required for the spontaneous development of destructive arthritis in mice deficient in IL-1 receptor antagonist. Proc Natl Acad Sci USA 2003;100:5986–90.
12. Lubberts E, Joosten LA, Oppers B, van den Bersselaar L, Coenen-de Roo CJ, Kolls JK, et al. IL-1-independent role of IL-17 in synovial inflammation and joint destruction during collagen-induced arthritis. J Immunol 2001;167:1004–13.
13. Lubberts E, Koenders MI, Oppers-Walgreen B, van den Bersselaar L, Coenen-de Roo CJ, Joosten LA, et al. Treatment with a neutralizing anti-murine interleukin-17 antibody after the onset of collagen-induced arthritis reduces joint inflammation, cartilage destruction, and bone erosion. Arthritis Rheum 2004;50:650–9.
14. Koenders MI, Lubberts E, Oppers-Walgreen B, van den Bersselaar L, Helsen MM, Di Padova FE, et al. Blocking of interleukin-17 during reactivation of experimental arthritis prevents joint inflammation and bone erosion by decreasing RANKL and interleukin-1. Am J Pathol 2005;167:141–9.
15. Koenders MI, Kolls JK, Oppers-Walgreen B, van den Bersselaar L, Joosten LA, Schurr JR, et al. Interleukin-17 receptor deficiency results in impaired synovial expression of interleukin-1 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.
16. Chabaud M, Durand JM, Miossec P. Human interleukin-17: a T cell-derived proinflammatory cytokine produced by the rheumatoid synovium. Arthritis Rheum 1999;42:963–70.
17. Kotake S, Udagawa N, Takahashi N, Matsuzaki K, Itoh K, Ishiyama S, et al. IL-17 in synovial fluids from patients with rheumatoid arthritis is a potent stimulator of osteoclastogenesis. J Clin Invest 1999;103:1345–52.
18. Ziolkowska M, Koc A, Maslinski W. 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. Chen YF, Jobanputra P, Barton P, Jowett S, Bryan S, Clark W et al. A systematic review of the effectiveness of adalimubub, etanercept and infliximab for the treatment of rheumatoid arthritis in adults and an economic exaluation of their cost-effectiveness. Health Technol Assess 2006;10:1–229.
20. Katz Y, Nadiv O, Beer Y. Interleukin-17 enhances tumor necrosis factor-α induced synthesis of interleukins 1,6, and 8 in skin and synovial fibroblasts: a possible role as a "fine-tuning cytokine" in inflammation processes. Arthritis Rheum 2001;44:2176–84.
21. LeGrand A, Fermor B, Fink C, Pisetsky DS, Weinberg JB, Vail TP, et al. Interleukin-1, tumor necrosis factor alpha, and interleukin-17 synergistically up-regulate nitric oxide and prostaglandin E2 production in explants of human osteoarthritic knee menisci. Arthritis Rheum 2001;44:2078–83.
22. Koenders MI, Lubberts E, van de Loo FA, Oppers-Walgreen B, van den Bersselaar L, Helsen MM, et al. Interleukin-17 acts independently of TNF-alpha under arthritic conditions. J Immunol 2006;176:6262–9.
23. Koenders MI, Joosten LA, van den Berg WB Potential new targets in arthritis therapy: interleukin (IL)-17 and its relation to tumour necrosis factor and IL-1 in experimental arthritis. Ann Rheum Dis 2006;65:29–33.
24. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988;31:315–24.
25. Honorati MC, Bovara M, Facchini A. Contribution of interleukin 17 to human cartilage degradation and synovial inflammation in osteoarthritis. Osteoarthritis Cartilage 2002;10:799–807.
26. Wonq PK, Quinn JM, Sims NA, van Nieuwenhuijze A, Campbell IK, Wicks IP, et al. Interleukin-6 modulates production of T lymphocyte-derived cytokines in antigen-induced arthritis and drives inflammation-induced osteoclastogenesis. Arthritis Rheum 2006;54:158–68.
27. Nishimoto N. Clinical benefits of anti-human IL-6 receptor antibody therapy. Clin Calcium 2007;17:562–8.
28. Mangan PR, Harrington LE, O'Quinn DB, Helms WS, Bullard DC, Elson CO, et al. Transforming growth factor-beta induces development of the T(H)17 lineage. Nature 2006;441:231–4.
29. Hirota K, Hashimoto M, Yoshitomi H, Tanaka S, Nomura T, Yamaguchi T, 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.

30. Bettelli E, Carrier Y, Gao W, Korn T, Strom TB, Oukka M, et al. Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. Nature 2006;441:235–8.
31. Murphy CA, Langrish CL, Chen Y, Blumenschein W, McClanahan T, Kastelein RA, et al. Divergent pro- and antiinflammatory roles for IL-23 and IL-12 in joint autoimmune inflammation. J Exp Med 2003;198:1951–7.
32. 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.
33. Bettelli E, Oukka M, Kuchroo VK. T(H)-17 cells in the circle of immunity and autoimmunity. Nat Immunol 2007;8:345–50.
34. Hirota K, Hashimoto M, Yoshitomi H, Tanaka S, Nomura T, Yamaguchi T, 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.
35. Gaffo A, Saag KG, Curtis JR. Treatment of rheumatoid arthritis. Am J Health Syst Pharm 2006;63:2451–65.
36. Mitoma H, Horiuchi T, Hatta N, Tsukamoto H, Harashima S, Kikuchi Y, et al. Infliximab induces potent anti-inflammatory responses by outside-to-inside signals through transmembrane TNF-α. Gastroenterology 2005;128:376–92.