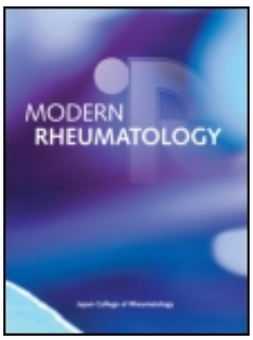

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ORIGINAL ARTICLE

Involvement of IL-33 in the pathogenesis of rheumatoid arthritis: the effect of etanercept on the serum levels of IL-33

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Abstract To investigate the role of interleukin (IL)-33 in rheumatoid arthritis (RA) patients, we measured the serum levels of IL-33 in RA patients before and after the administration of etanercept. Twenty-four patients with RA were treated with etanercept. Clinical and laboratory examinations, including serum levels of C-reactive protein (CRP) and hemoglobin (Hb); white blood cell (WBC) and red blood cell (RBC) counts; and the Disease Activity Score of 28 joints including CRP (DAS28-CRP), were performed at the baseline and at 3 and 6 months after the initial treatment with etanercept. The mean serum IL-33 levels had decreased significantly at 3 and 6 months after the initial treatment with etanercept. Serum IL-33 levels showed a significant correlation with the number of tender joints, CRP, DAS28-CRP, and the WBC count, and an inverse correlation with the RBC count and Hb level. These findings indicated that the decrease of serum IL-33 levels was a novel function of etanercept, shown for the first time in this study. Measurement of serum levels of IL-33 may become a useful control marker for RA treatment.

Keywords Rheumatoid arthritis · Interleukin-33 · Etanercept

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Introduction

Rheumatoid arthritis (RA) is characterized by chronic inflammatory disease, including synovial proliferation and excessive proinflammatory cytokine production, resulting in cartilage and bone destruction [1–4]. Recently, successful results have been obtained in RA treatment with the targeting of cytokines, including tumor necrosis factor (TNF)-α. The production of several cytokines is involved in joint destruction in patients with RA [5–8]. TNF-α, interleukin (IL)-1, IL-6, IL-8, and IL-15 are especially critical in forming the inflammatory process of RA [5–7]. Blocking of the action of these cytokines such as that produced by the recent use of TNF-α or IL-6 blockers is essential for the regulation of joint destruction in patients with RA. IL-33 was recently identified as a ligand for the IL-1 family receptor T1/ST2 [9] and has been broadly detected in various tissues, including the stomach, lung, spinal cord, brain, skin, and smooth muscle. The synovial tissues of the joints in RA patients and arthritic mice expressed IL-33 production [10], but it remains obscure whether this new cytokine, IL-33, affects the progression of joint destruction of RA in vivo. Some reports suggest a strong relationship between joint inflammation and IL-33 in RA [11, 12]. In addition, TNF-α has been reported to stimulate the production of IL-33 in vitro [13]. Therefore,

when a TNF-α blocker is used for the treatment of RA, the therapeutic effect may depend on whether TNF-α induces the production of IL-33 in vivo. In the present study, to investigate the clinical role of IL-33 in RA patients, we measured the IL-33 levels in RA patients before and after the administration of a TNF-α blocker, etanercept.

Patients, materials, and methods

Measurements of serum IL-33 levels in RA patients treated with etanercept and healthy control individuals

Twenty-four patients (22 females and 2 males; mean age 60.7 ± 9.0 years; mean disease duration 12.9 ± 7.4 years; Steinbrocker's stage I: 7, II: 8, III: 8, IV: 1) with RA, diagnosed according to the criteria of the American College of Rheumatology, participated in this study between October 2009 and December 2010. All patients in this study were treated with etanercept for more than 6 months. The treatment with etanercept was continued in all patients for the term of surveilance in the study. Three patients also received continual treatment with prednisolone (mean dose 0.50 ± 1.35 mg/day). Etanercept was administered by subcutaneous injection at a dose of 25 mg once a week. We usually administer etanercept by injections at this dose and frequency. Six patients received etanercept in combination with methotrexate (given perorally at a constant dose of 4–8 mg/week). Eighteen patients were treated with etanercept alone. In addition, nine healthy controls matched for age (mean age 62.2 ± 8.3 years) were studied. Informed consent for study participation was obtained from all participants in accordance with the ethics regulations of Heisei General Hospital.

Clinical and laboratory evaluations

Clinical and laboratory examinations, including serum levels of C-reactive protein (CRP), anti-agalactosyl IgG antibody (CA-RF), and hemoglobin (Hb); white blood cell (WBC) and red blood cell (RBC) counts; scoring on the visual analogue scale (VAS); and examination for the Disease activity score of 28 joints including CRP (DAS28-CRP), were performed at the baseline and at 3 and 6 months after the initial treatment with etanercept. Serum samples were obtained from the RA patients just before the initial injection of etanercept at the baseline and at 3 and 6 months after the initial treatment with etanercept and stored at −80°C until assayed. The serum levels of IL-33 were measured in all patients, as described below. Nine healthy subjects matched for age were used as a control group for the serum levels of IL-33.

Measurements of serum levels of IL-33

The levels of IL-33 in serum from RA patients with etanercept treatment and healthy individuals were measured using an enzyme-linked immunosorbent assay (ELISA), following the manufacturer's instructions (Human IL-33 ELISA kits; Komabiotech, Seoul, Korea). All samples were measured in duplicate.

Statistical analysis

In this study, all data are shown as means ± standard deviation. In the statistical analysis, we used the Wilcoxon signed rank test to compare the values measured before and after etanercept treatment. The matching of the ages between RA patients and control individuals was assessed by using the Bartlett test. Single regression analysis was performed between serum IL-33 and clinical laboratory parameters, and Pearson's correlation coefficients were determined. In this study, we considered p values of less than 0.05 to be significant. All statistical analysis was performed using the Statcel II software program.

Results

Clinical and laboratory disease activity

CRP levels decreased significantly, from 3.26 ± 3.23 mg/dl at pretreatment to 0.87 ± 0.89 mg/dl ($p < 0.001$) and 0.70 ± 0.82 mg/dl ($p < 0.001$) at 3 and 6 months, respectively, after the initial injection of etanercept (Table 1). The numbers of swollen joints and tender joints, VAS, CRP level, DAS28-CRP, and WBC count were significantly decreased at 3 and 6 months compared with values at pretreatment.

Serum levels of IL-33 in RA patients before and after treatment with etanercept

To investigate whether the ELISA system we used was sensitive to rheumatoid factor (RF), we measured the levels of serum or SF from RA patients after the addition of human IgG for the absorption of RF. The addition of human IgG (1 mg/ml) and absorption of RF did not lead to a statistically significant difference in the IL-33 levels (data not shown). The mean serum IL-33 levels in RA patients receiving etanercept treatment decreased significantly, from 820 ± 867 pg/ml at pretreatment to 502 ± 550 pg/ml ($p < 0.01$) and 398 ± 414 pg/ml ($p < 0.001$) at 3 and 6 months, respectively, after the initial injection of

Table 1 Changes in clinical and laboratory parameters in patients with rheumatoid arthritis (RA) treated with etanercept

	Etanercept group			Control group
	Baseline	3 M	6 M	
Number of swollen joints	6.14 ± 3.66	2.57 ± 2.56*	1.93 ± 2.16***	ND
Number of tender joints	2.57 ± 2.17	0.79 ± 1.42**	0.50 ± 1.34**	ND
VAS (mm)	51.1 ± 16.3	24.1 ± 8.6**	19.8 ± 9.1***	ND
CRP (mg/dl)	3.26 ± 3.23	0.87 ± 0.89***	0.70 ± 0.82***	ND
DAS 28-CRP	3.76 ± 0.82	2.26 ± 0.63**	1.96 ± 0.59**	ND
Serum anti-AG IgG (AU/ml)	230 ± 284	224 ± 331	187 ± 290	ND
WBC (/μl)	7120 ± 2070	5970 ± 1280*	5860 ± 1100**	ND
RBC ($\times 10^4$/μl)	409 ± 46	411 ± 55	426 ± 33	ND
Hb (g/dl)	11.55 ± 1.27	11.78 ± 1.84	12.08 ± 0.79	ND
Serum IL-33 (pg/ml)	820 ± 867	502 ± 550**	398 ± 414***	177 ± 172#

Data values are shown as means ± SD. Data at 3 or 6 months (M) were compared with those at baseline

ND not done, *VAS* visual analogue scale, *DAS 28-CRP* disease activity score of 28 joints including C-reactive protein, *anti-AG IgG* anti-agalactosyl IgG antibody *Hb* hemoglobin

* $p < 0.05$ versus baseline, ** $p < 0.01$ versus baseline, *** $p < 0.001$ versus baseline

# $p < 0.05$ versus control value

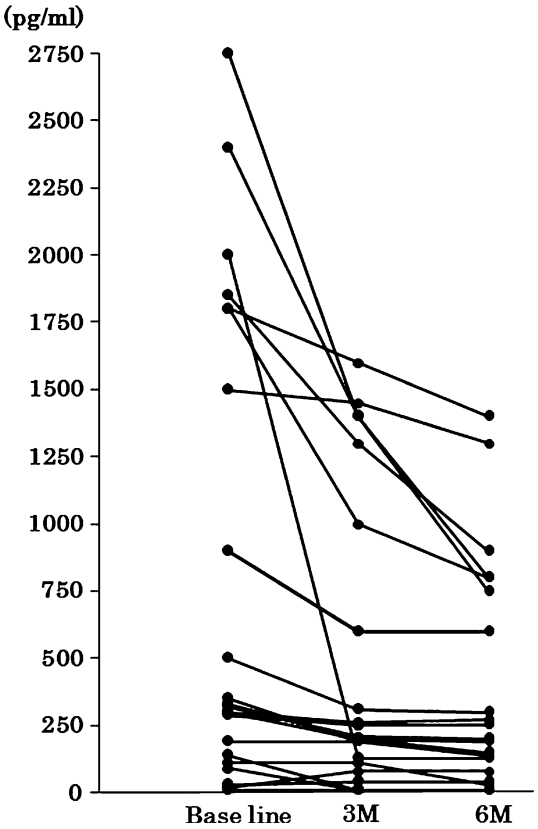

Fig. 1 Serum interleukin (IL)-33 levels in RA patients at pretreatment and 3 and 6 months after initial treatment with etanercept

etanercept (Table 1) (Fig. 1). The mean serum baseline level of IL-33 in RA patients was higher than that in control individuals ($p < 0.05$).

Correlation analysis between serum IL-33 and clinical laboratory parameters

Serum IL-33 levels showed significant correlations with the number of tender joints ($r = 0.307$, $p < 0.05$), VAS ($r = 0.376$, $p < 0.05$), CRP level ($r = 0.684$, $p = 0.0001$), DAS28-CRP ($r = 0.357$, $p < 0.05$), and the WBC count ($r = 0.450$, $p < 0.05$) (Table 2). In addition, serum IL-33 levels showed an inverse correlation with the RBC count and Hb levels.

Discussion

TNF-α inhibitors can reduce the levels of a variety of cytokines and chemokines that may form the TNF-α cascade in RA patients [14–17]. To investigate the in vivo action mechanism of etanercept, we measured the serum levels of IL-33 in RA patients treated with etanercept. The serum IL-33 levels were significantly reduced by the treatment with etanercept. This indicated a novel function of etanercept, although it is not yet clear whether IL-33 truly affects the disease activity in RA patients in vivo. The results of a recent study indicated that infliximab reduced the serum IL-33 levels in RA patients [18]. These findings, along with those of the present study, suggest that IL-33 is involved in the cytokine cascade of TNF-α in vivo. IL-33 expression has been observed in the synovial membrane, and endothelial cells in the inflamed synovium appear to be a major source of IL-33 [19]. In a collagen-induced arthritis (CIA) model, the administration of a soluble IL-33 receptor (sST2) had a protective effect against the

Table 2 Correlations between serum interleukin (IL)-33 levels and other laboratory parameters in RA patients who received etanercept treatment

	Correlation coefficient	*p*
Number of swollen joints	0.0532	0.7411
Number of tender joints	0.3072	0.0498*
VAS	0.3755	0.0156*
CRP	0.6837	0.0001**
DAS28-CRP	0.3565	0.0222*
WBC	0.4502	0.0162*
RBC	−0.6604	0.0001**
Hb	−0.7062	0.0001**

* $p < 0.05$, ** $p < 0.001$

progression of arthritis [20]. In other studies, the administration of IL-33 exacerbated antigen-induced arthritis by activating mast cells [11] and autoantibody-induced arthritis [12]. These reports suggest a role of IL-33 in the progression of RA.

RA is generally considered to be a Th1-dominant disease, and Th2 cytokines are thought to attenuate the activity of RA. There are reports that the administration of IL-33 induced the production of IL-4, IL-5, and IL-13, indicating the potential of IL-33 to induce Th2 responses [9, 21]. On the other hand, it has been reported that a Th2-biased cytokine pattern develops in RA patients prior to the onset of RA, or at least very early during the development of the disease [22, 23]. The marked decrease in interferon (IFN)-γ production brought about by draining lymph node (LN) cells from anti-ST2 antibody-treated mice with CIA suggests that IL-33 is also involved in the development of Th1 responses [10]. Smithgall et al. [24] reported that IL-33 amplified both Th1- and Th2-type responses in basophiles and invariant natural killer (iNK) T cells. Th1 and Th2 responses following IL-33 treatment may depend on the kind of stimulation, the cell type, and the cytokine environment.

Receptor activator of nuclear factor-kappa B ligand (RANKL) mRNA expression was reduced in the joints of anti-ST2-treated mice, suggesting that anti-ST2 treatment affects local as well as systemic responses and may beneficially affect RANK-mediated bone erosions [10]. This may indicate the role of IL-33 in the development of bone destruction in RA. In our study, serum IL-33 levels were significantly correlated with clinical parameters of RA, including the number of tender joints, DAS28-CRP, CRP levels, and the WBC count. This finding may indicate an intimate relationship between IL-33 and the inflammatory status of RA. The measurement of serum IL-33 levels in RA patients may become a useful control marker for RA treatment.

Interestingly, several stimuli, such as TNF-α and hypoxia, increase IL-33 expression [13]. Palmer et al. [10] observed that cultured synovial fibroblasts produced IL-33, and that IL-33 expression was increased in response to IL-1β and/or TNF-α. On the other hand, intraarticular injection of IL-33 in mice also induced the production of TNF-α [25]. Treatment with ST2-Fc also modulated the immune response, decreasing IFN-γ and TNF-α production of spleen cells upon restimulation with type II collagen in vitro. Therefore, we need to investigate the existence of an autocrine cascade between IL-33 and TNF-α in vivo.

In future, IL-33 might become a target for the treatment of RA. When biologics that target IL-33 are developed, the role of IL-33 in inflammatory processes in RA will become clear. The decrease of IL-33 levels may be a useful marker with which to assess the efficacy of etanercept in the treatment of RA.

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Conflict of interest None.

References

1. McInnes IB, Schett G. Cytokines in the pathogenesis of rheumatoid arthritis. Nat Rev Immunol. 2007;7:429–42.
2. Janossy G, Panayi G, Duke O, Bofill M, Poulter LW, Goldstein G. Rheumatoid arthritis: a disease of T-lymphocyte/macrophage immunoregulation. Lancet. 1981;17(2):839–42.
3. Feldmann M, Brennan FM, Maini RN. Role of cytokines in rheumatoid arthritis. Annu Rev Immunol. 1996;14:397–440.
4. Ulfgren AK, Lindblad S, Klareskog L, Andersson J, Andersson U. Detection of cytokine producing cells in the synovial membrane from patients with rheumatoid arthritis. Ann Rheum Dis. 1995;54:654–61.
5. Paleolog EM, Young S, Stark AC, McCloskey RV, Feldmann M, Maini RN. Modulation of angiogenic vascular endothelial growth factor by tumor necrosis factor alpha and interleukin-1 in rheumatoid arthritis. Arthritis Rheum. 1998;41:1258–65.
6. Feldmann M, Maini RN. Anti-TNF alpha therapy of rheumatoid arthritis: what have we learned? Annu Rev Immunol. 2001;19:163–96.
7. Kageyama Y, Takahashi M, Torikai E, Suzuki M, Ichikawa T, Nagafusa T, Koide Y, Nagano A. Treatment with anti-TNF-alpha antibody infliximab reduces serum IL-15 levels in patients with rheumatoid arthritis. Clin Rheumatol. 2007;26:505–9.
8. Kageyama Y, Ichikawa T, Nagafusa T, Torikai E, Shimazu M, Nagano A. Etanercept reduces the serum levels of interleukin-23 and macrophage inflammatory protein-3 alpha in patients with rheumatoid arthritis. Rheumatol Int. 2007;28:137–43.
9. Schmitz J, Owyang A, Oldham E, Song Y, Murphy E, McClanahan TK, et al. IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. Immunity. 2005;23:479–90.
10. Palmer G, Talabot-Ayer D, Lamacchia C, Toy D, Seemayer CA, Viatte S, et al. Inhibition of interleukin-33 signaling attenuates

the severity of experimental arthritis. Arthritis Rheum. 2009;60: 738–49.
11. Xu D, Jiang HR, Kewin P, Li Y, Mu R, Fraser AR, et al. IL-33 exacerbates antigen-induced arthritis by activating mast cells. Proc Natl Acad Sci USA. 2008;105:10913–8.
12. Xu D, Jiang HR, Li Y, Pushparaj PN, Kurowska-Stolarska M, Leung BP, et al. IL-33 exacerbates autoantibody-induced arthritis. J Immunol. 2010;184:2620–6.
13. Wood IS, Wang B, Trayhurn P. IL-33, a recently identified interleukin-1 gene family member, is expressed in human adipocytes. Biochem Biophys Res Commun. 2009;384:105–9.
14. Ichikawa T, Kageyama Y, Kobayashi H, Kato N, Tsujimura K, Koide Y. Etanercept treatment reduces the serum levels of interleukin-15 and interferon-gamma inducible protein-10 in patients with rheumatoid arthritis. Rheumatol Int. 2010;30: 725–30.
15. Kageyama Y, Kobayashi H, Kato N. Infliximab treatment reduces the serum levels of interleukin-23 in patients with rheumatoid arthritis. Mod Rheumatol. 2009;19:657–62.
16. Kageyama Y, Kobayashi H, Kato N, Shimazu M. Etanercept reduces the serum levels of macrophage chemotactic protein-1 in patients with rheumatoid arthritis. Mod Rheumatol. 2009;19: 372–8.
17. Musacchio E, Valvason C, Botsios C, Ostuni F, Furlan A, Ramonda R, et al. The tumor necrosis factor-alpha-blocking agent infliximab inhibits interleukin 1 beta (IL-1beta) and IL-6 gene expression in human osteoblastic cells. J Rheumatol. 2009;36:
1575–9.
18. Mu R, Huang HQ, Li YH, Li C, Ye H, Li ZG. Elevated serum interleukin 33 is associated with autoantibody production in patients with rheumatoid arthritis. J Rheumatol. 2010;37:2006–13.
19. Carriere V, Roussel L, Ortega N, Lacorre DA, Americh L, Aguilar L, et al. IL-33, the IL-1-like cytokine ligand for ST2 receptor, is a chromatin-associated nuclear factor in vivo. Proc Natl Acad Sci USA. 2007;104:282–7.
20. Leung BP, Xu D, Culshaw S, McInnes IB, Liew FY. A novel therapy of murine collagen-induced arthritis with soluble T1/ST2. J Immunol. 2004;173:145–50.
21. Rank MA, Kobayashi T, Kozaki H, Bartemes KR, Squillace DL, Kita H. IL-33-activated dendritic cells induce an atypical TH2-type response. J Allergy Clin Immunol. 2009;123:1047–54.
22. Kokkonen H, Soderstrom I, Rocklov J, Hallmans G, Lejon K, RantapaaDahlqvist S. Up-regulation of cytokines and chemokines predates the onset of rheumatoid arthritis. Arthritis Rheum. 2010;62:383–91.
23. Raza K, Falciani F, Curnow SJ, Ross EJ, Lee CY, Akbar AN, et al. Early rheumatoid arthritis is characterized by a distinct and transient synovial fluid cytokine profile of T cell and stromal cell origin. Arthritis Res Ther. 2005;7:784–95.
24. Smithgall MD, Comeau MR, Yoon BR, Kaufman D, Armitage R, Smith DE. IL-33 amplifies both Th1- and Th2-type responses through its activity on human basophils, allergen-reactive Th2 cells, iNKT and NK cells. Int Immunol. 2008;20:1019–30.
25. Verri WA Jr, Souto FO, Vieira SM, Almeida SC, Fukada SY, Xu D, et al. IL-33 induces neutrophil migration in rheumatoid arthritis and is a target of anti-TNF therapy. Ann Rheum Dis. 2010;69:1697–703.