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The role of IL-12 in inflammatory activity of patients with rheumatoid arthritis (RA)

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SUMMARY

The aim of this study was to investigate the role of IL-12 in patients with RA. IL-12 (p70) and its associated cytokines were measured in sera and synovial fluid (SF) using an enzyme-linked immunosorbent method. Seven American College of Rheumatology (ACR) core set measures as well as IL-12 levels were sequentially monitored at the commencement and 4 months after treatment with a low-dose steroid and disease-modifying anti-rheumatic drugs (DMARDs). In sera, 64 (42·2%) of 152 RA patients had detectable concentrations of IL-12 (p70), whereas one (1·4%) of 69 osteoarthritis (OA) patients and five (10%) of 50 healthy controls had detectable IL-12 ($P < 0{\cdot}001$). The median level of circulating IL-12 was also higher in RA patients ($P < 0{\cdot}001$). In SF, the number of patients with detectable IL-12 and the median IL-12 levels were significantly higher in RA patients ($n = 53$) than in OA patients ($n = 22$). In paired samples ($n = 53$) of sera and SF from RA patients, IL-12 levels were higher in the SF than in sera ($P < 0{\cdot}001$). Patients with detectable IL-12 ($n = 51$) in sera had higher tender joint scores ($P = 0{\cdot}003$), swollen joint scores ($P < 0{\cdot}001$) and C-reactive protein (CRP; $P = 0{\cdot}036$), than those without ($n = 55$). Four months after treatment with DMARDs, the improved group showed a larger IL-12 decrease than the non-improved group ($P = 0{\cdot}017$). The levels of IL-12 correlated positively with those of IL-2, interferon-gamma, IL-6, and tumour necrosis factor-alpha, but were correlated inversely with those of IL-10. Our results demonstrate that IL-12 levels reflect RA disease activity and that IL-12 is involved in the production of proinflammatory cytokines. An IL-12 blockade could be useful for the treatment of RA.

Keywords IL-12 disease activity rheumatoid arthritis

INTRODUCTION

RA is an autoimmune disease characterized by the proliferation of synovium and the infiltration of chronic inflammatory cells. Cytokines from synovium and inflammatory cells are thought to be important in the initiation and perpetuation of RA. In particular, macrophage-derived cytokines**,** including tumour necrosis factor-alpha (TNF-α) and IL-6, have been detected at high levels and many of these monokines function as potent proinflammatory molecules in the joints, and reflect the disease activity of RA [1–5]. Recently, the imbalance of cytokines originating from T lymphocytes has been raised as an issue in the pathogenesis of RA [6,7]. CD4 T lymphocytes could be functionally classified into T helper 1 (Th1), which secrete IL-2 and interferon-gamma (IFN-γ), and T helper 2 (Th2), which secrete IL-4, IL-5 and IL-10 [6,7]. In experimental animal models of arthritis it is known that Th1 lymphocytes induce a pathogenic response, whereas Th2 lymphocytes induce a protective response [8–11].

IL-12 is now recognized as a critical cytokine in terms of regulating the balance between Th1 and Th2 cells**,** as well as enhancing cytotoxic T cell-mediated lysis and natural killer (NK) cell activity [12]. It is composed of the p35 and p40 subunits, neither of which has been found to display any significant biological function alone [13]. Instead, a heterodimeric form of IL-12, p70, mediates a biological response, whereas a p40 homodimer acts as an IL-12 antagonist [14,15]. In recent years IL-12 has been shown to play a critical role in the regulation of immune responses in various autoimmune disease models. The administration of anti-IL-12 antibodies eliminates established inflammation in experimental murine colitis [16]. The severity of experimental autoimmune encephalitis is increased by the systemic administration of recombinant IL-12 and prevented by antibodies to IL-12 [17]. Likewise, IL-12 in combination with type II collagen induces

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severe arthritis in DBA/1 mice [18]. In a number of the published reports, it has been suggested that IL-12 plays a role in the pathogenesis of T cell-mediated autoimmune disease.

It has been documented that IL-12 synergizes with a variety of cytokines and induces the production of IFN-γ and proinflammatory cytokines [19–21]. On the other hand, IL-12-induced IFN-γ production is down-regulated by IL-4 and IL-10 [22,23]. In patients with RA, IL-12 p70 induces IFN-γ dominant cytokine production by infiltrating T cells into chronic arthritic joints *in vitro*, suggesting that IL-12 may play an important role in a shift toward rheumatoid T cells with Th1 cytokine profiles [24]. However, the association of IL-12 with the other cytokines *in vivo* has not been clearly documented in RA. In addition, the role played by IL-12, if any, remains to be defined in human RA. We hypothesized that the enhanced expression of IL-12 in RA would lead to the production of proinflammatory cytokines and that this would be reflected in RA disease activity. To investigate this hypothesis, we measured IL-12 in the sera and the synovial fluid (SF) of RA patients, and compared it with the clinical and laboratory parameters of RA disease activity, and to the other cytokines known to be involved in the pathogenesis of RA [1–11].

PATIENTS AND METHODS

Patients

One hundred and fifty-two patients who fulfilled the revised criteria of the American Rheumatism Association for RA were involved in this study [25]. The mean age of the RA patients (32 males and 125 females) was 48 years (range 22–74 years). The mean disease duration was 78·4 months (range 1–396 months). Comparisons were made with 69 patients with osteoarthritis (OA; eight male and 61 females) and 50 healthy controls (seven males and 43 females) with no rheumatic diseases. The mean age of the OA patients and the healthy controls was 53 years (range 38–72 years) and 46 years (range 21–63 years), respectively. No differences were found in age or sex between RA, OA and healthy controls.

Clinical and laboratory assessments

In patients with RA, clinical assessments were performed at commencement and 4 months after treatment by one rheumatologist, and one nurse trained in making standardized assessments. Each patient's condition was assessed by the same investigator throughout the study. Seven clinical variables (American College of Rheumatology (ACR) core set measures) were evaluated, as defined by the ACR [26]: tender joint count, swollen joint count, pain as recorded on a 100-mm visual–analogue scale, physician's global assessment of disease activity, patient's global assessment of disease activity, degree of disability, as measured by a Health Assessment Questionnaire, erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP). Rheumatoid factor (RF) was measured using nephelometry and was considered positive when the results were > 20 U/ml.

Cytokine measurements

Sera were taken from RA patients in the early morning and at the same time of day (at commencement and at 4 months after treatment) clinical assessments were performed, and stored at −20°C in a refrigerator. SF from RA patients with joint effusions was also collected by arthrocentesis. IL-12 (p70), IL-2, IFN-γ, TNF-α, IL-6, IL-4 and IL-10 in sera and SF were measured using cytokine-specific ELISAs, as previously described [27]. Briefly, cytokine measurement was as follows: 4 μg/ml of MoAbs to human cytokines (R&D Systems Inc., Minneapolis, MN) were added to a 96-well plate (Nunc, Roskilde, Denmark) and incubated overnight at 4°C. After incubating the plate with blocking solution (PBS containing 1% bovine serum albumin (BSA) and 0·05% Tween 20) for 2 h at room temperature, the patient's sera diluted 1:2 and the standard recombinant cytokines (R&D Systems) were added to the 96-well plate and incubated at room temperature for 2 h. After washing four times with PBS containing Tween 20, 500 ng/ml of biotinylated MoAbs to human cytokines (R&D Systems) were added and the reactions were allowed to proceed for 2 h at room temperature. After washing, 2000-fold diluted streptavidin–alkaline–phosphate (Sigma Bioscience, St Louis, MO) was added, and the reaction was again allowed to proceed for 2 h. After washing four times, 1 mg/ml of *p*-nitrophenylphosphate (Sigma Bioscience) dissolved in diethanolamine (Sigma Bioscience) was added to induce the colour reaction. NaOH (1 N; Fisher Scientific, Pittsburgh, PA) was used to stop the reaction. An automated microplate reader (Vmax; Molecular Devices, Palo Alto, CA) at 405 nm was used to measure the optical density (OD). IL-6 was measured using the commercially available ELISA kit (Endogen Inc., Woburn, MA) according to the manufacturer's instructions. The sensitivities of IL-2 and IL-10 were 10 pg/ml. The sensitivities of IL-12, IFN-γ, TNF-α, IL-6 and IL-4 were 5 pg/ml. ODs below the detection limit for the assay were presented as the values on linear regression made from ODs of the serially diluted standard cytokines.

Statistical analysis

Because the various data sets were not normally distributed, results were expressed as medians (minimum, maximum). Comparisons of numerical data between the groups were performed using the Mann–Whitney rank sum test or the Kruskal–Wallis test, and of category-based data comparisons using either the χ^2 test or Fisher's exact probability test, where appropriate. Correlation between two variables was performed using Spearman's rank correlation coefficient. $P < 0{\cdot}05$ was considered statistically significant.

RESULTS

Levels of IL-12 in sera and synovial fluid

In sera, 64 (42·2%) of 152 RA patients had detectable concentrations (> 5 pg/ml) of IL-12 p70, whereas one (1·4%) of the 69 OA patients and five (10%) of the 50 healthy controls had detectable IL-12 ($P < 0{\cdot}001$) (Table 1). The median level of circulating IL-12 was significantly higher in RA patients than in both OA patients and healthy controls (median (range) 2·7 (0, 170·8) pg/ml in RA, 0·4 (0, 9·5) pg/ml in OA, 0 (0, 10·9) pg/ml in healthy controls; $P < 0{\cdot}001$). In SF, the number of patients with detectable IL-12 and the median IL-12 level were also higher in RA patients ($n = 53$) than in OA patients ($n = 22$) (the number of patients with detectable IL-12: 54·7% *versus* 13·6%, $P = 0{\cdot}001$; median level: 7·4 pg/ml *versus* 0 pg/ml; $P < 0{\cdot}001$). In the paired samples of sera and SF from RA, IL-12 levels were significantly higher in the SF than in the sera ($P < 0{\cdot}001$) (Fig. 1). However, the levels of IL-12 were not different between the sera and SF in patients with OA ($P =$ NS).

Correlation of IL-12 with disease activity

Patients with RA ($n = 106$), in whom clinical assessment was

Table 1. Levels of IL-12 in sera and synovial fluids obtained from patients with RA and osteoarthritis (OA) and healthy controls

IL-12 (pg/ml)	RA	OA	Healthy controls	*P*
Sera	$n=152$	$n=69$	$n=50$	
Positivity* (no.)	42·2% (64)	1·4% (1)	10% (4)	<0·001
Median (range), pg/ml	2·7 (0, 170·8)	0·4 (0, 9·5)	0 (0, 10·9)	<0·001
Synovial fluid	$n=53$	$n=22$		
Positivity* (no.)	52·8% (23)	13·6% (3)	–	0·001
Median (range), pg/ml	7·4 (0, 155·6)	0 (0, 9·3)	–	<0·001

*The percentage of patients or controls with detectable levels of IL-12 (>5 pg/ml).

performed at base line, were divided into two groups: patients with detectable concentrations of IL-12 in sera ($n=51$, median (range) of IL-12 29·5 (5·0, 116·9) pg/ml) and those without ($n=55$, median (range) of IL-12 0 (0, 4·9) pg/ml). The above mentioned seven categories of disease activity of RA were then compared for the two groups. There were no differences in age, sex, disease duration, pain score, patient's global assessment, physician's global assessment, patient's self-assessed disability, and ESR between the two groups (Table 2). However, patients with detectable IL-12 had a higher tender joint score ($P=0·003$), swollen joint score ($P<0·001$) and CRP ($P=0·036$), compared with those without. Levels of IL-12 correlated well with the tender joint score ($r=0·469$, $P<0·001$), swollen joints score ($r=0·453$, $P<0·001$), visual analogue pain scale ($r=0·279$, $P=0·005$), physician's global assessment ($r=0·267$, $P=0·008$), patient's global assessment ($r=0·231$, $P=0·02$), and CRP ($r=0·238$, $P=0·014$) (Fig. 2). However, no correlation was found between IL-12 levels and the degree of disability or ESR.

Sequential measurement of circulating IL-12 in patients with RA

In 57 patients with RA, the above mentioned seven categories were sequentially monitored at commencement and 4 months after treatment with prednisone, a non-steroidal anti-inflammatory drug and disease-modifying anti-rheumatic drugs (DMARDs) including methotrexate, anti-malarial drugs, sulfasalazine, bucillamine, and gold. Of 57 patients, 26 (46·5%) were improved by treatment, using the improvement definition suggested by the ACR [28]. At base line, there were no differences in age, sex, disease duration, kinds of DMARD, the amount of prednisone, above mentioned seven categories, and IL-12 levels between the improved ($n=26$) and the non-improved groups ($n=31$) (Tables 3 and 4). However, the number of patients treated with two or more kinds of DMARD was significantly higher in the improved group (73·1% *versus* 41·9%, $P=0·018$). Four months after treatment, IL-12 levels were significantly decreased in the improved group ($P<0·001$), but not in the non-improved group ($P=$ NS) (Fig. 3). The extent of IL-12 decrease was greater in the improved group than in the

Table 2. Comparison of clinical and laboratory parameters between patients with detectable IL-12 in sera and those without

Variables*	Patients with detectable IL-12† ($n=51$)	Patients without detectable IL-12 ($n=55$)	*P*
Age, year	48 (22, 72)	50 (23, 68)	NS
Female/male, number	45/6	49/6	NS
Disease duration, months	78·3 (3, 298)	72·3 (1, 226)	NS
Tender joint score	23 (2, 68)	12 (0, 68)	0·003
Swollen joint score	12 (1, 62)	5 (0, 50)	<0·001
Visual analogue pain scale, mm	52 (0, 100)	49 (0, 100)	NS
Physician's global assessment	5·0 (0, 10·0)	4·8 (0, 9·3)	NS
Patient's global assessment	5·0 (0, 10·0)	4·8 (0, 10·0)	NS
Degree of disability	1·5 (1·0, 3·6)	1·4 (1·0, 3·1)	NS
Erythrocyte sedimentation rate, mm/h	43·0 (5·0, 133·0)	38·0 (3·4, 122·0)	NS
C-reactive protein, mg/*l*	21·0 (3·3, 122·0)	10·3 (3·1, 148)	0·036
Rheumatoid factor titres, U/ml	115·0 (10·6, 2460·0)	71·8 (9·1, 676·0)	0·09

*Data are presented as median (minimum, maximum) except male/female numbers and the number of patients with detectable IL-12. Possible scores ranged from 0 to 68 for number of tender joint; from 0 to 66 for the number of swollen joint; from 1 (very good) for the global assessment; from 0 (no pain) to 100 (maximal pain imaginable) for pain, measured on 100-mm visual analogue scale; and from 0 (no disability) to 3 (high disability) for degree of disability. Health Assessment Questionnaire Measures are based on Outcome Measures in Rheumatoid Arthritis Clinical Trials [26]. NS, Not significant.

†Patients with detectable concentrations of circulating IL-12 (>5 pg/ml).

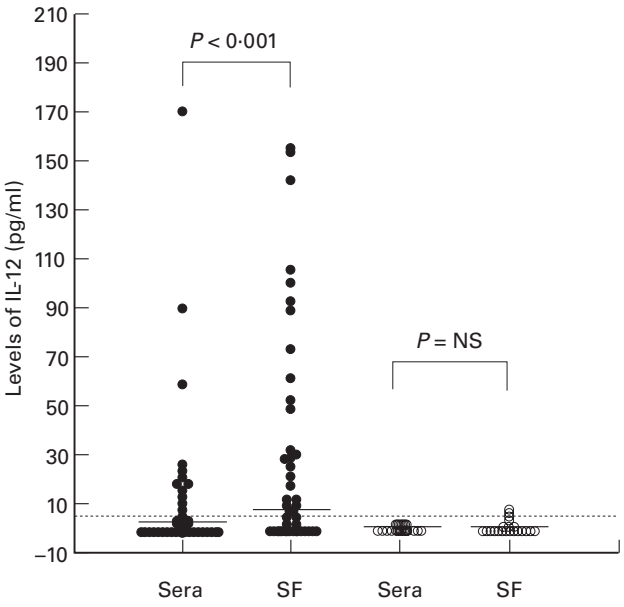

Fig. 1. The levels of IL-12 in paired sera and synovial fluid (SF) obtained simultaneously from patients with RA (●; $n = 53$) and osteoarthritis (○; OA) ($n = 22$). Broken line indicates the limit of detectable concentration of IL-12 (5 pg/ml). Bars represent median levels. Comparison of median IL-12 levels between sera and SF was performed using paired Wilcoxon signed ranks test. NS, Not significant.

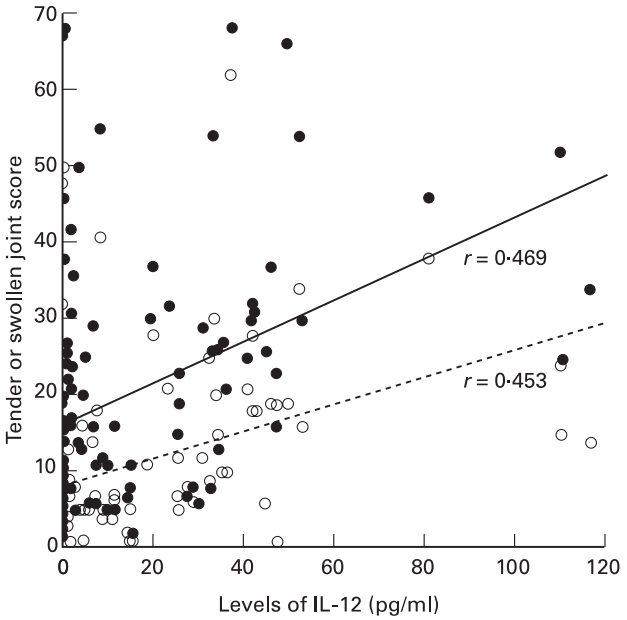

Fig. 2. Correlations of IL-12 levels with tender or swollen joint scores in 104 patients with RA. ——, ●, Tender joint scores; - - - - -, ○, swollen joint scores.

non-improved group together with the amelioration of the clinical parameters for disease activity (change of IL-12: median −5·6 pg/ml *versus* 0 pg/ml, $P = 0.017$ (+ denotes increase, − denotes decrease)) (Table 4).

Correlations of IL-12 with other cytokines

To investigate the association between IL-12 and the other cytokines known to be involved in the pathogenesis of RA [1–15], we measured IL-2, IFN-γ, TNF-α, IL-6, IL-4 and IL-10 levels in the sera and SF on samples collected at the same time as IL-12 levels were determined. In sera, patients ($n = 36$) with detectable IL-12 (> 5 pg/ml) had higher median levels of TNF-α ($P = 0·006$) and IL-6 ($P < 0·001$) and a lower median level of IL-10 ($P = 0·001$) than those ($n = 58$) without (Table 5). Circulating IL-12 levels correlated positively with IL-6 ($r = 0·432$, $P < 0·001$) and TNF-α ($r = 0·315$, $P = 0·002$), whereas an inverse correlation was found with IL-10 ($r = -0·323$, $P = 0·002$) (data not shown). In SF, patients ($n = 23$) with detectable IL-12 had higher median levels of IL-2 ($P = 0·011$), IFN-γ ($P < 0·001$), TNF-α ($P = 0·007$), and IL-6 ($P = 0·002$) and tended to have a lower median level of IL-10 ($P =$ NS) than those ($n = 30$) without (Table 5). IL-12 in the

Table 3. Comparison of demographics and the medications used between improved and non-improved RA patients at base line

Characteristics	Improved patients ($n = 26$)	Non-improved patients ($n = 31$)	*P*
Age, years*	45 (25, 67)	49 (27, 71)	NS
Female/male, number	23/3	28/3	NS
Disease duration, months*	57·2 (1, 186)	65·3 (6, 292)	NS
Dose of prednisone, mg/day*	5 (0, 10)	5 (0, 10)	NS
Percent treated with:			
Methotrexate	80·8	70·1	NS
Anti-malarial	50·0	41·9	NS
Sulfasalazine	26·9	19·4	NS
Bucillamine	15·3	6·5	NS
Gold	15·3	6·5	NS
No. of second-line drugs, %			
> 3	15·3	3·2	NS
> 2	73·1	41·9	0·019

*Data are presented as median (minimum, maximum). NS, Not significant.

Table 4. Comparison of outcome measures and circulating IL-12 between improved and non-improved group

Outcome measures and IL-12 levels	Baseline		Change at 4 months*		
	Improved ($n=26$)	Non-improved ($n=3$ 1)	Improved ($n=26$)	Non-improved ($n=31$)	P†
Tender joint count	25 (7, 68)	22 (5, 68)	−9 (−29, 6)	3 (−11, 17)	<0·001
Swollen joint count	10 (4, 34)	6 (0, 50)	−5 (−30, 6)	0 (−36, 19)	<0·001
Pain score (VAS), mm	51 (15, 100)	51 (0, 78)	−10 (−67, 50)	4 (−39, 49)	0·004
Patient's global assessment	4·9 (1·2, 10)	5·0 (0, 8·7)	−1·1 (−7·0, 6·3)	0·4 (–3·6, 5·9)	0·014
Physician's global assessment	5·0 (1·6, 7·8)	4·7 (0, 9·2)	−0·9 (−5·1, 5·0)	0·4 (–5·9, 6·4)	0·07
Patient's self-assessed disability	1·5 (1·0, 3·1)	1·5 (1·0, 3·1)	0 (–1·5, 2·9)	0 (−1·6, 2·3)	NS
Erythrocyte sedimentation rate, mm/h	41 (20, 92)	33 (6, 122)	−14·5 (−88, 35)	1 (−109, 133)	<0·001
C-reactive protein, mg/*l*	17·5 (3·4, 162)	10·3 (3·3, 80·2)	−8·8 (−134, 7·9)	−0·1 (−46, 27·4)	0·001
IL-12, pg/ml	8·3 (0·6, 116·9)	11·7 (0, 53·4)	−5·6 (−116·9, 13·7)	0 (−48·3, 110·8)	0·017

*Negative values indicate decreases and positive values increases in variables measured at 4 months, compared with base line.

†Significance of differences in the improved *versus* non-improved group, at 16 weeks. At base line, no significant differences were found in outcome measures and IL-12 levels between improved and non-improved group. NS, Not significant.

SF correlated well with IL-6 ($r=0·449$, $P=0·001$), TNF-α ($r=0·481$, $P<0·001$), and IFN-γ ($r=0·369$, $P=0·007$) (Fig. 4).

DISCUSSION

The role of IL-12 has been addressed in the context of the pathogenesis of RA. The administration of IL-12 enhanced disease expression and severity in an animal model of RA [17]. However, a blockade of IL-12 during the induction of collagen-induced arthritis markedly attenuated the severity of arthritis [29,30]. It has also been documented that IL-12 is highly expressed by infiltrating macrophage and synovial lining cells in patients with RA [31]. Patients with RA have significantly higher levels of IL-12 p70, a biologically active form of IL-12, in the sera and SF, compared with OA patients and healthy controls [32,33]. However, this was not confirmed by another study [34]. Therefore, it remained to be defined whether p70 heterodimer, rather than p40 homodimer, is elevated in RA and thus plays a role in the pathogenesis.

In the present study, the largest undertaken to date to examine IL-12 heterodimer in RA, p70 levels were significantly higher in sera and SF of patients with RA than those of patients with OA or healthy controls, which confirms previous reports [32,33]. In addition, in paired samples of the sera and SF, IL-12 p70 levels were significantly higher in SF than sera in patients with RA, but there was no difference between sera and SF in patients with OA. These observations suggest that the production of IL-12 is concentrated at the actual site of the immune reaction. Excessive production of IL-12 in the joints could play a role in the pathogenesis of RA.

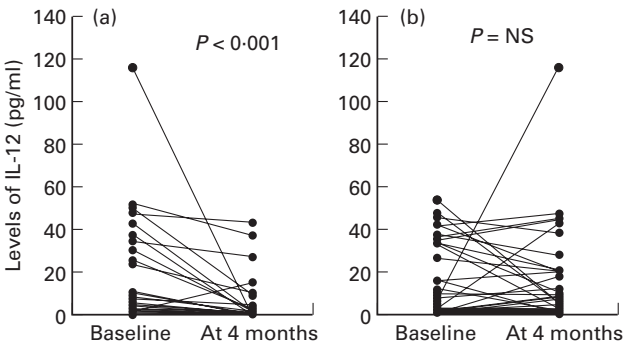

Fig. 3. Changes of IL-12 levels 4 months after treatment compared with baseline in (a) improved ($n=26$) *versus* (b) non-improved patients ($n=31$) with RA.

It has also been reported that elevated levels of circulating IL-12 are associated with the activity or severity of various autoimmune diseases. In patients with juvenile chronic arthritis, for example, the serum concentrations of total IL-12 are significantly elevated in clinically active patients compared with inactive patients and healthy controls [35]. In patients with active multiple sclerosis (MS), serum levels of IL-12 are detectable in 53%, whereas none of the patients with clinically inactive MS has detectable IL-12 [36]. Moreover, IL-12 levels correlate well with the degree of inflammation in cerebrospinal fluid [37]. In patients with diabetes mellitus (DM), higher levels of IL-12 p70 are detected in both insulin-dependent and -independent DM than are found in healthy controls, and these correlate well with the presence of retinopathy [38,39]. RA resembles the above mentioned diseases in that T cells, possibly Th1 cells, play an important role in the pathogenesis. We demonstrated first, that patients with detectable levels of IL-12 in sera had higher tender joint scores, swollen joint scores, and CRP. Moreover, IL-12 levels correlated well with several parameters indicative of disease activity in RA, especially with the tender and swollen joint scores. These observations suggest that IL-12 production may be associated with an exacerbation of RA, and then circulating IL-12 levels could be useful to assess disease activity.

Immunosuppressive agents such as cyclosporin and FK 506 are known to inhibit the production of IL-12 *in vitro* [40,41], but the effect of DMARDs, commonly used in RA, such as methotrexate, hydroxychloroquine, and sulfasalazine on the production of IL-12 is little documented. We found that after treatment, circulating IL-12 was significantly decreased in parallel with an amelioration of disease activity in improved RA patients. The only difference in base line characteristics between improved and non-improved patients was the number of DMARDs used. These findings suggest that DMARDs might have a potential to decrease IL-12 production and that an IL-12 blockade could be of therapeutic benefit in

Table 5. Comparisons of the other cytokine levels between patients with detectable IL-12 and those without

Cytokines (pg/ml)	Patients with detectable IL-12	Patient without detectable IL-12	*P**
Sera	$n=36$	$n=58$	
TNF-α	44·3 (0, 310·5)	25·6 (0, 241·8)	0·006
IL-6	78·0 (0, 332·0)	1·2 (0, 309·3)	<0·001
IL-10	34·8 (3·0, 97·4)	49·5 (13·5, 119·1)	0·001
Synovial fluid	$n=28$	$n=25$	
IL-2	23·5 (0, 76·3)	14·0 (0, 59·9)	0·011
IFN-γ	30·2 (12·4, 43·6)	21·1 (5·9, 38·1)	<0·001
TNF-α	63·7 (0, 356·3)	17·9 (0, 213·8)	0·007
IL-6	119·7 (0, 569·5)	13·3 (0, 233·4)	0·002
IL-4	2·7 (0, 37·6)	2·9 (0, 53·7)	NS
IL-10	47·7 (29·1, 714·2)	54·8 (14·8, 134·4)	NS

*Significance of differences in RA patients with detectable IL-12 (>5 pg/ml) *versus* those without. Patients with detectable IL-12 had higher levels of IL-2, IFN-γ, tumour necrosis factor-alpha (TNF-α) and IL-6 in the synovial fluid, but lower levels of IL-10 in the sera compared with those without. Data are presented as median (minimum, maximum). NS, Not significant.

human RA. However, we are not able to conclude here which drugs affected IL-12 production because no comparative study was performed. Further *in vitro* studies using mononuclear cells or *ex vivo* studies are required to resolve this issue.

IL-12 synergizes with IL-2 and TNF-α in the production of IFN-γ, whereas IL-12-induced IFN-γ production is suppressed by IL-4 and IL-10 [19–23]. Small amounts of IL-12 also can induce the production of proinflammatory cytokines, including IL-1 and TNF-α, which contribute to the signs and symptoms of RA. Moreover, their levels correlate well with activity markers of RA [1–3]. IL-12 also augments the ability of rheumatoid synovial T cells to produce IFN-γ [24]. However, there are few *in vivo* studies which show that IL-12 correlates with the other pro-inflammatory cytokines in RA. In the present study, IL-12 levels in the sera and SF positively correlated with TNF-α and IL-6 levels, suggesting that IL-12 production is closely linked to the production of proinflammatory cytokines, which is also supported by the correlation between IL-12 and several parameters indicative of disease activity. In addition, IL-12 positively correlated with IL-2 and IFN-γ, but correlated inversely with IL-10, which is consistent with previous reports that IL-12 may play a critical role in the differentiation of naive $CD4^+$ T cells towards an IFN-γ-producing Th1-type cell in RA [24,34]. These observations suggest that patients with elevated IL-12 may have a marked or increased level of disease activity and more activated macrophages, which may produce more detectable cytokines, by immune cell interactions.

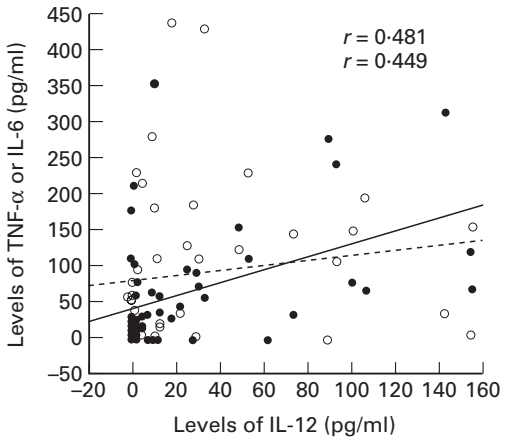

Fig. 4. Correlations of IL-12 levels with IL-6 and tumour necrosis factor-alpha (TNF-α) levels in synovial fluid of RA ($n=53$). ———, ●, TNF-α; - - - - -, ○, IL-6.

In conclusion, IL-12 p70 was elevated in the sera and SF and correlated well with RA disease activity. After treatment with DMARDs, IL-12 levels were decreased in parallel with a clinical improvement. IL-12 correlated positively with IL-2, IFN-γ, IL-6, and TNF-α, whereas it correlated inversely with IL-10. Our results suggest that IL-12 reflects the disease activity of RA and that it may be involved in the production of proinflammatory cytokines. An IL-12 blockade could be useful for the treatment of RA.

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REFERENCES

1 Brennan FM, Maini RN, Feldmann M. TNF-alpha: pivotal role in rheumatoid arthritis? Br J Rheumatol 1992; **31**:293–8.
2 Dayer JM, Beutler B, Cerami A. Cachectin/tumor necrosis factor stimulates collagenase and prostaglandin E2 production by human synovial cells and dermal fibroblasts. J Exp Med 1985; **162**:2163–8.
3 Tetta C, Camussi G, Modena V, Di Vittorio C, Baglioni C. Tumour necrosis factor in serum and synovial fluid of patients with active and severe rheumatoid arthritis. Ann Rheum Dis 1990; **49**:665–7.
4 Guerne PA, Zuraw BL, Vaughan JH, Carson DA, Lotz M. Synovium as a source of interleukin 6 in vitro. Contribution to local and systemic manifestations. J Clin Invest 1989; **83**:585–92.
5 Ito A, Itoh Y, Sasaguri Y, Morimatsu M, Mori Y. Effects of interleukin-6 on the metabolism of connective tissue components in rheumatoid synovial fibroblasts. Arthritis Rheum 1992; **35**:1197–201.
6 Mosmann TR, Coffman RL. Heterogenecity of cytokine secretion patterns and functions of helper T cells. Adv Immunol 1989; **46**:111–47.
7 Romagnani S. Human Th1 and Th2 subsets: doubt or more. Immunol Today 1991; **12**:256–7.
8 Shehadeh NN, Larosa F, Lafferty KJ. Altered cytokine activity in adjuvant inhibition of autoimmune diabetes. J Autoimmun 1993; **6**:291–300.
9 Matyniak JE, Reiner SL. T helper phenotype and genetic susceptibility in experimental Lyme disease. J Exp Med 1995; **181**:1251–4.
10 Racke MK, Bonomo A, Scott DE, Cannella B, Levine A, Raine CS, Shevach EM, Rocken M. Cytokine-induced immune deviation as a therapy for inflammatory autoimmune disease. Cytokine-induced immune deviation as a therapy for inflammatory autoimmune disease. J Exp Med 1994; **180**:1961–6.
11 Kuchroo VK, Das MP, Brown JA *et al*. B7-1 and B7-2 costimulatory molecules activate differentially the Th1/Th2 development pathways: application to autoimmune disease therapy. Cell 1995; **80**:707–18.
12 Trinchieri G. Interleukin-12: a proinflammatory cytokine with immunoregulatory functions that bridge innate resistance and antigen-specific adaptive immunity. Annu Rev Immunol 1995; **13**:251–76.
13 Gubler U, Chua AO, Schoenhaut DS *et al*. Coexpression of two distinct genes is required to generate secreted bioactive cytotoxic lymphocyte maturation factor. Proc Natl Acad Sci USA 1991; **88**:4143–7.

14 Ling P, Gately MK, Gubler U *et al*. IL-12 p40 homodimer binds to the IL-12 receptor but does not mediate biologic activity. J Immunol 1995; **154**:116–27.
15 Gillessen S, Carvajal D, Ling P *et al*. Mouse interleukin-12 (IL-12) p40 homodimer: a potent IL-12 antagonist. Eur J Immunol 1995; **25**:200–6.
16 Ehrhardt RO, Ludviksson BR, Gray B, Neurath M, Strober W. Induction and prevention of colonic inflammation in IL-2 deficient mice. J Immunol 1997; **158**:566–73.
17 Leonard JO, Waldburger KE, Goldman SJ. Prevention of experimental autoimmune encephalomyelitis by an antibodies against interleukin 12. J Exp Med 1995; **181**:381–6.
18 Germann T, Szeliga J, Hess H, Storkel S, Podlaski FJ, Gately MK, Schmitt E, Rude E. Administration of interleukin 12 in combination with type II collagen induces severe arthritis in DBA/1 mice. Proc Natl Acad Sci USA 1995; **92**:4823–7.
19 Gazzinelli RT, Heiny S, Wynn TA, Wolf S, Sher A. Interleukin 12 is required for the T lymphocyte-independent induction of interferon-γ by an intracellular parasite and induces resistance in T cell-deficient host. Proc Natl Acad Sci USA 1993; **90**:6115–9.
20 Germann T, Gately MK, Schoenhaut DS *et al*. Interleukin 12/T cell stimulating factor, a cytokine with multiple effects on T helper type 1 (Th1) but not on Th2 cells. Eur J Immunol 1993; **23**:1762–70.
21 Nastala CL, Edington HD, McKinney TG *et al*. Recombinant IL-12 administration induces tumor regression in association with IFN-γ production. J Immunol 1994; **153**:1676–706.
22 D'Andrea A, Aste-Amezega M, Valiante NM, Ma X, Kubin M, Trinchieri G. Interleukin 10 (IL-10) inhibits human lymphocyte interferon γ production by suppressing natural killer cell stimulatory factor/IL-12 synthesis in accessory cells. J Exp Med 1993; **178**: 1041–8.
23 Kiniwa M, Gately M, Gubler U, Chizzonite R, Fargeas C, Delespesse G. Recombinant interleukin-12 suppresses the synthesis of IgE by interleukin-4 stimulated human lymphocytes. J Clin Invest 1992; **90**:262–6.
24 Morita Y, Yamamura M, Nishida K *et al*. Expression of interleukin-12 in synovial tissue from patients with rheumatoid arthritis. Arthritis Rheum 1998; **41**:306–14.
25 Arnett FC, Edworthy SM, Bloch DA *et al*. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988; **31**:315–24.
26 Felson DT, Anderson JJ, Boers M *et al*. The American College of Rheumatology preliminary core set of disease activity measures for rheumatoid arthritis clinical trials. Arthritis Rheum 1993; **36**:729–40.
27 Abrams JS, Roncarolo MG, Yssel H, Andersson U, Gleich GJ, Silver JE. Strategies of anti-cytokine monoclonal antibody development: immunoassay of IL-10 and IL-5 in clinical samples. Immunol Rev 1992; **127**:5–24.
28 Felson DT, Anderson JJ, Boers M *et al*. American College of Rheumatology preliminary definition of improvement in rheumatoid arthritis. Arthritis Rheum 1995; **38**:727–35.
29 Malfait AM, Butler DM, Presky DH, Maini RN, Brennan FM, Feldmann M. Blockade of IL-12 during the induction of collagen-induced arthritis (CIA) markedly attenuates the severity of the arthritis. Clin Exp Immunol 1998; **111**:377–83.
30 McIntyre KW, Shuster DJ, Gillooly KM *et al*. Reduced incidence and severity of collagen-induced arthritis in interleukin-12-deficient mice. Eur J Immunol 1996; **26**:2933–8.
31 Sakkas LI, Johanson NA, Scanzello CR, Platsoucas CD. Interleukin-12 is expressed by infiltrating macrophages and synovial lining cells in rheumatoid arthritis and osteoarthritis. Cell Immunol 1998; **188**:105–10.
32 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.
33 Bucht A, Larsson P, Weisbrot L *et al*. Expression of interferon-gamma (IFN-gamma), IL-10, IL-12 and transforming growth factor-beta (TGF-beta) mRNA in synovial fluid cells from patients in the early and late phases of rheumatoid arthritis (RA). Clin Exp Immunol 1996; **103**:357–67.
34 Wang F, Sengupta TK, Zhong Z, Ivashkiv LB. Regulation of the balance of cytokine production and the signal transducer and activator of transcription (STAT) transcription factor activity by cytokines and inflammatory synovial fluids. J Exp Med 1995; **182**:1825–31.
35 Gattorno M, Picco P, Vignola S, Stalla F, Buoncompagni A, Pistoia V. Serum interleukin 12 concentration in juvenile chronic arthritis. Ann Rheum Dis 1998; **57**:425–8.
36 Drulovic J, Mostarica-Stojkovic M, Levic Z *et al*. Serum interleukin-12 levels in patients with multiple sclerosis. Neurosci Lett 1998; **251**:129–32.
37 Fassbender K, Ragoschke A, Rossol S *et al*. Increased release of interleukin-12p40 in MS: association with intracerebral inflammation. Neurology 1998; **51**:753–8.
38 Winkler G, Dworak O, Salamon F, Salamon D, Speer G, Cseh K. Increased interleukin-12 plasma concentrations in both insulin-dependent and non-insulin-dependent diabetes mellitus. Diabetologia 1998; **41**:488.
39 Rabinovitch A, Suarez-Pinzon WL, Sorensen O. Interleukin 12 mRNA expression in islets correlates with beta-cell destruction in NOD mice. J Autoimmun 1996; **9**:645–51.
40 Bonnotte B, Pardoux C, Bourhis H *et al*. Inhibition of the human allogeneic mixed lymphocyte response by cyclosporine A: relationship with IL-12 pathway. Tissue Antigens 1996; **48**:265–70.
41 Degiannis D, Koniavitou K. In vitro expression of activation markers and lymphocyte proliferation in response to interleukin-12: effect of immunosuppressive agents. Transplant Proc 1996; **28**:3062–4.