Interleukin 10 Treatment of Patients with Rheumatoid Arthritis Enhances Fcγ Receptor Expression on Monocytes and Responsiveness to Immune Complex Stimulation

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ABSTRACT. ***Objective.*** Several clinical studies performed with human recombinant interleukin 10 (IL-10) in patients with rheumatoid arthritis (RA) have shown little efficacy. We investigated potentially proinflammatory *in vivo* effects of IL-10 in humans. We evaluated the upregulation of Fcγ receptor (FcγR) expression on monocytes/macrophages (and granulocytes) in patients with RA receiving different dosages of IL-10.

Methods. Together with changes in disease activity and several cell markers, the expression of FcγRI, FcγRIIa, and FcγRIII was determined on granulocytes and monocytes/macrophages from the peripheral blood of 6 patients with active RA before and after treatment with recombinant human IL-10. In addition, the *in vitro* effect of IL-10 on FcγR expression on monocytes/macrophages in combination with their susceptibility to immune complex induced production of tumor necrosis factor-α (TNF-α) was assessed.

Results. Clinical improvement was not observed in the IL-10 treated patients (based on ACR20 criteria). Significant decreases in thrombocyte numbers were observed in patients receiving IL-10. No changes in cell markers such as CD14 were found. On the other hand, expression of FcγRI and FcγRIIa on monocytes/macrophages was increased upon high dose IL-10 treatment. Interestingly, increases in expression of FcγRI and FcγRIIa correlated with a decrease in thrombocyte numbers. *In vitro*, IL-10 similarly upregulated FcγRI and FcγRIIa expression on monocytes/macrophages from RA patients. This was accompanied by increased TNF-α production after immune complex stimulation.

Conclusion. These findings indicate that upregulation of FcγR expression in RA with IL-10 treatment may counteract the otherwise antiinflammatory effects of IL-10 by potentiating immune complex mediated proinflammatory responses. (J Rheumatol 2003;30:648–51)

Key Indexing Terms:
RHEUMATOID ARTHRITIS CLINICAL TRIAL INTERLEUKIN 10
Fcγ RECEPTORS IMMUNE COMPLEXES TUMOR NECROSIS FACTOR-α

In chronically inflamed joints of patients with rheumatoid arthritis (RA), substantial amounts of interleukin 10 (IL-10) are produced. IL-10 is considered to temper ongoing proinflammatory responses in these patients[1]. In different experimental arthritis models and in a number of human *in vitro* studies, IL-10 inhibited inflammatory activity[2,3]. Apparently, the amount of intraarticularly produced IL-10 in RA patients is insufficient to adequately control the disease. For this reason clinical studies were designed to treat RA patients with IL-10. In contrast to the unambiguous antiinflammatory effects of IL-10 in many experimental conditions, IL-10 treatment of RA patients (rhuIL-10, phase I) resulted in very little or no clinical benefit[4]. This apparent inconsistency may be due to an ambivalent role of IL-10 in being not only antiinflammatory, but possibly also proinflammatory[3].

In vitro, IL-10 enhances expression of Fcγ on monocytes/macrophages[5]. Through these upregulated IgG receptors, immune complexes can stimulate proinflammatory and tissue destructive activity of monocytes and macrophages[6,7]. Since immune complexes are present in significant amounts in RA patients[8], this may specifically stimulate monocyte/macrophage activity. We evaluated Fcγ receptor (FcγR) regulation in patients with active RA receiving IL-10.

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Effects on monocyte/macrophage activity were measured *in vitro* using RA peripheral blood mononuclear cells exposed to IL-10 and immune complexes.

MATERIALS AND METHODS

Clinical study. Within a multicenter clinical dose-finding study (phase II/III, double blind, placebo controlled), 6 patients with RA with active disease were studied at our hospital. Patients received subcutaneously either placebo twice weekly (n = 1), 4 µg/kg IL-10 daily (n = 1), 8 µg/kg IL-10 twice weekly (n = 2), or 8 µg/kg daily (n = 2). Disease activity was assessed by measuring a broad range of disease variables, including C-reactive protein (CRP) levels, erythrocyte sedimentation rate (ESR), and number of tender and swollen joints, at baseline, at one week, and every 2 weeks up to 6 weeks.

Peripheral blood monocytes (CD14+) and granulocytes (CD66b+) in whole blood were stained at baseline and at 6 weeks for FcγRI, FcγRIIa, and FcγRIII, and complement receptor 3 (CR3) with FITC conjugated murine anti-human monoclonal antibodies (clones 32.2, IV.3, and 3G8, respectively, all provided by Medarex, Annandale, NJ, USA). Clone IV.3 was previously shown to preferentially recognize FcγRIIa and not IIb[9]. Expression of cell markers was assessed by flow cytometry (FACScan, Becton Dickenson) and analysis was done using WinMDI software[9].

In vitro study. The *in vitro* effects of IL-10 on monocyte FcγR expression and responsiveness to immune complexes were tested on peripheral blood mononuclear cells (PBMC) of 8 RA patients. PBMC (5×10^6/ml) were cultured for 24 h in 96 well, round bottom plates in the absence or presence of IL-10 (10 ng/ml). Apart from assessment of FcγR by double staining and flow cytometry (see above), for each donor a portion of the cells was washed thoroughly, counted, and replated in 24 well flat bottom plates (5×10^5/ml). Immune complexes (preformed) were than added in different concentrations (0.01, 0.1, and 1 µg/ml) to the PBMC and cultured for 3 days. After this period supernatants were collected, rendered cell-free, and stored below –20°C. TNF-α and IL-1ß were measured with ELISA (Biosource Europe, Nivelles, Belgium). The immune complexes were prepared by incubation of 200 µg/ml ovalbumin (Sigma, St. Louis, MO, USA) and 400 µg/ml polyclonal rabbit anti-ovalbumin (Sigma) for 20 min at 37°C.

Statistical analysis. The Wilcoxon signed rank test for paired observations was used to compare *ex vivo* and *in vitro* FcγR expression on CD14+ cells and cytokine production. Correlations were evaluated with Spearman correlation analysis. A p value < 0.05 was considered statistically significant.

RESULTS

Clinical study. An increase in disease activity was observed in the patient who received placebo. A decrease in CRP and the number of tender and swollen joints was observed in the patient receiving the lowest dose of IL-10. However, no IL-10 treated patient improved by more than 20% according to the American College of Rheumatology response criteria[10]. Further, none of the disease variables was significantly altered by IL-10 treatment (data not shown). Patients receiving higher IL-10 dosages (8 µg/kg, n = 4) showed increased CRP levels (from 59 ± 33 at baseline to 111 ± 77 at 6 weeks, p = 0.068; Figure 1c). White blood cell counts and rheumatoid factor did not change significantly.

In the IL-10 treated patients FcγRI and IIa expression of monocytes increased (both $p < 0.05$, n = 5, Figure 1A, B). Expression of FcγRIII remained low and was not changed significantly by IL-10. Granulocyte FcγRI, FcγRIIa, and FcγRIII expression levels [at baseline, mean fluorescence intensities (MFI) were 12 ± 12, 80 ± 8, and 1119 ± 391, respectively; n = 5] were also not significantly altered with IL-10 treatment (at 6 weeks MFI were 15 ± 8, 78 ± 14, and 1249 ± 453, respectively). Increases in FcγR were not due to changes in cell size, since this was not significantly changed (data not shown). Specificity of the response was confirmed because other surface molecules such as CD14 and CR3 were not significantly changed (MFI 344 ± 38 vs 352 ± 35 and MFI 96 ± 29 vs 104 ± 22 at baseline and after 6 weeks for CD14 and CR3, respectively; n = 5).

None of the disease variables (ESR, CRP, and numbers of swollen and tender joints) correlated with changes in FcγR. Only a decrease in thrombocyte numbers was observed in patients receiving IL-10 ($p < 0.05$ at 1 and 2 weeks; n = 5) (Figure 2A). This decrease was sustained at least for 6 weeks in the high dose IL-10 group and correlated significantly with increased expression of monocytic FcγRI ($r = –0.94$, $p < 0.01$) and FcγRIIa ($r = –0.95$, $p < 0.01$) (Figure 2B). No correlation of thrombocytes with changes in expression of FcγRIII was found.

In vitro study. Similar to the *in vivo* results, IL-10 (10 ng/ml) *in vitro* specifically increased FcγRI and FcγRIIa expression of monocytes (both $p < 0.01$, Figure 3A). FcγRIII expression on monocytes was not changed by IL-10. Also, no change in FcγR expression on lymphocytes was observed (data not shown). The IL-10 primed monocytes with enhanced FcγRI and FcγRIIa expression were more easily stimulated by immune complexes (1 µg/ml, not with 0.01 and 0.1 µg/ml) to produce TNF-α (Figure 3B). IL-1ß production was also measured but was below the detection limit (< 20 pg/ml) in all cultures.

DISCUSSION

Our data are the first to show that treatment of RA patients with IL-10 *in vivo* results in a specific increase of monocyte FcγR. These observations are in accord with the effects of IL-10 on monocytes *in vitro*, as we have shown here, confirming previous reports[5]. We also found IL-10 primed RA monocytes to be more susceptible to immune complex stimulation, resulting in increased TNF-α production. Considering the prominent role of TNF-α in proinflammatory responses and joint destruction in RA, this suggests that priming of monocytes by IL-10 can enhance their proinflammatory potential[3,11]. The priming concentration we chose for the *in vitro* study is within the range of IL-10 serum levels measured in healthy individuals with IL-10 treatment[12]. Further, the duration of IL-10 incubation *in vitro* may match the *in vivo* situation, since levels of IL-10 peak after 2 hours and have largely disappeared after 24 hours[12]. This indicates that priming conditions *in vivo* mimic those induced *in vitro*. These observations suggest that upregulation of FcγR expression upon IL-10 treatment may counteract the otherwise antiinflammatory activity of IL-10,

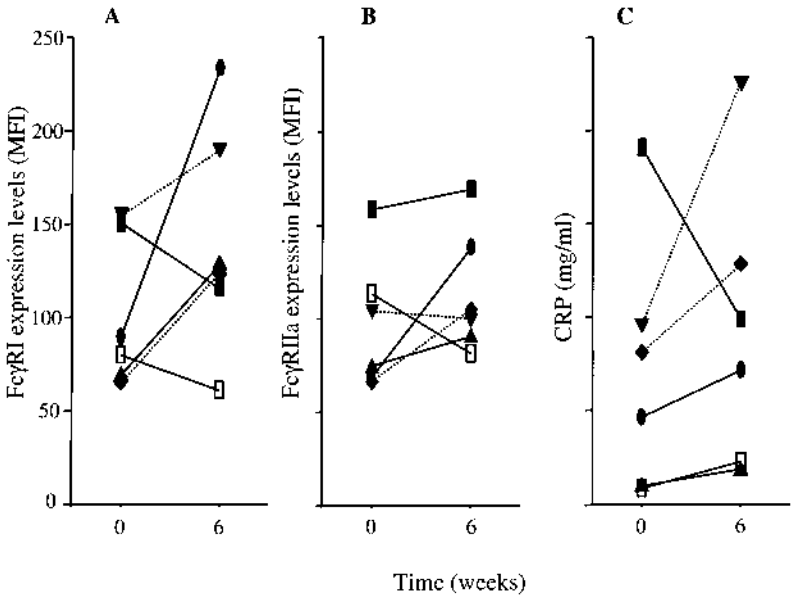

Figure 1. Changes in FcγRI and IIa expression levels (A, B; MFI: mean fluorescence intensity) and CRP levels (C) after 6 weeks of IL-10 treatment compared to baseline expression. Treatments consisted of placebo (n = 1, □), 4 µg/kg daily (■, n = 1), 8 µg/kg twice a week (broken lines, ▼, ◆, n = 2), or 8 µg/kg daily (solid lines, ▲, ●, n = 2). Individual patients are indicated by different symbols. The average expression of FcγRI and FcγRIIa on monocytes after 6 weeks of IL-10 therapy (n = 5 patients) was statistically significantly increased compared to baseline (both $p < 0.05$).

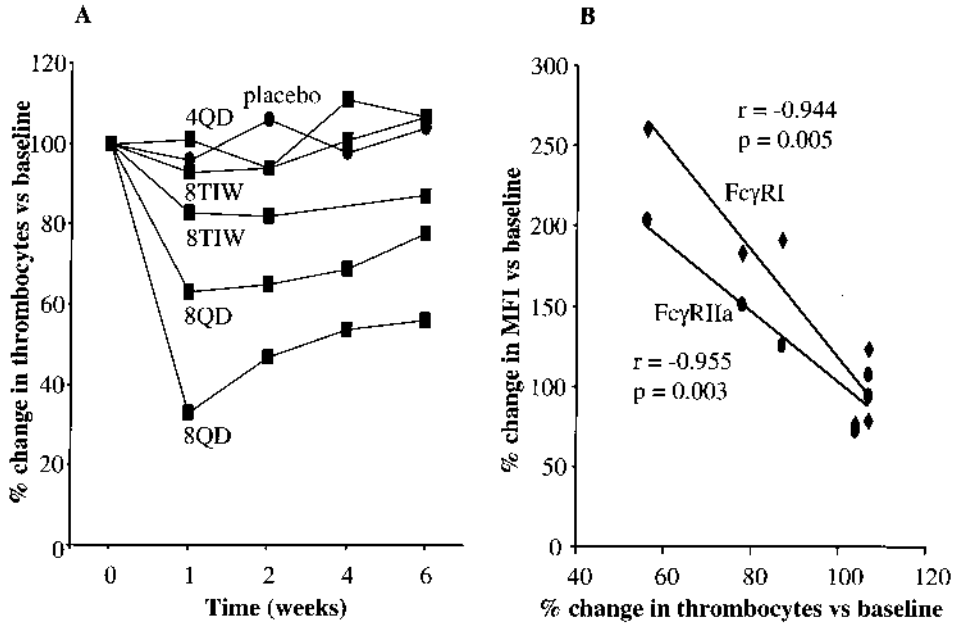

Figure 2. A. Percentage of change in thrombocyte levels (at 1, 2, 4, and 6 wks) compared to baseline ($410 \times 10^9 \pm 117.10^9$/l). Treatments consisted of placebo (n = 1, ●), 4 µg/kg daily (4QD, n = 1), 8 µg/kg twice a week (8TIW, n = 2), or 8 µg/kg daily (8QD, n = 2). B. Changes in thrombocytes correlated with expression of FcγRI and FcγRIIa 6 weeks after treatment compared to baseline. Correlation coefficients (r) and p values (p) are given.

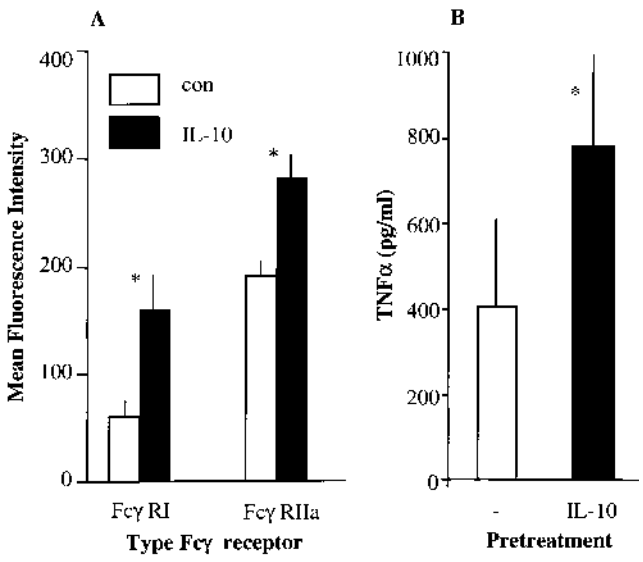

Figure 3. A. Average expression (MFI) of FcγRI and FcγRIIa on monocytes after 24 h of *in vitro* culture with IL-10 (10 ng/ml, n = 8). B. Effect of ovalbumin-anti-ovalbumin immune complexes on TNF-α secretion by untreated or IL-10 pretreated monocytes from RA patients. Immune complexes (1 µg/ml) induced TNF-α production to a higher extent in IL-10 pretreated monocytes than in non-pretreated monocytes. *p < 0.05 vs control culture.

by potentiating immune complex mediated proinflammatory responses and tissue destruction in RA.

The cause of the decreased thrombocyte numbers with IL-10 treatment in RA patients is unknown, but may reflect immune complex/FcγR mediated events involving monocytes/macrophages. In human FcγRIIa-transgenic mice, antibody-binding thrombocytes that express FcγRIIa are effectively cleared by FcγRIIa-expressing monocytes/macrophages[13]. The observation that thrombocyte-monocyte complexes are increased in patients with RA compared to healthy controls indicates that such interactions can occur in RA patients[14]. Immune complexes (in particular those containing rheumatoid factor) present in RA patients may link FcγR-expressing monocytes to FcγRIIa-expressing thrombocytes and facilitate clearance of thrombocytes. Interestingly, with respect to these findings, it was observed that IL-4, which in contrast to IL-10 downregulates FcγRI, FcγRIIa, and FcγRIII, increased thrombocyte levels in RA patients[3,15].

FcγR upregulation on monocytes may represent an undesirable side effect of IL-10 in treatment of autoimmune diseases like RA, since this counteracts the otherwise anti-inflammatory properties of this cytokine, as shown in healthy individuals without immune complexes[12]. Downregulation of FcγR expression during IL-10 treatment (e.g., by IL-4[15]) may therefore improve clinical efficacy in the treatment of RA and other autoimmune diseases with enhanced FcγR mediated proinflammatory events.

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