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. In different experimental arthritis models and in a number of human in vitro studies, IL-10 inhibited inflammatory activity. 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. This apparent inconsistency may be due to an ambivalent role of IL-10 in being not only antiinflammatory, but possibly also proinflammatory.

In vitro, IL-10 enhances expression of Fcγ on monocytes/macrophages. Through these upregulated IgG receptors, immune complexes can stimulate proinflammatory and tissue destructive activity of monocytes and macrophages. Since immune complexes are present in significant amounts in RA patients, this may specifically stimulate monocyte/macrophage activity. We evaluated Fcγ receptor (FcγR) regulation in patients with active RA receiving IL-10.
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 and FcγRIII remained low and was not changed at 6 weeks, p = 0.068; Figure 1c). White blood cell counts increased CRP levels (from 59 ± 33 at baseline to 111 ± 77 at 6 weeks). 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 FcγRIIa 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 × 10^9 ± 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.
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. 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. 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.

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.

Downregulation of FcγR expression during IL-10 treatment may therefore improve clinical efficacy in the treatment of RA and other autoimmune diseases with enhanced FcγR mediated proinflammatory events.

REFERENCES