

Treatment of rheumatoid arthritis patients with anti-TNF- α monoclonal antibody is accompanied by down-regulation of the activating Fc γ Receptor I on monocytes

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Abstract

Objectives

To study the effect of anti-TNF- α therapy on activating IgG Fc receptor (Fc γ R) expression on monocytes of RA patients in relation to changes in disease activity.

Methods

RA patients were treated with anti-TNF- α mAb (infliximab). At baseline, 2 and 14 weeks after the start of anti-TNF- α treatment, Fc γ R expression levels on circulating monocytes were evaluated. Changes in expression were correlated to changes in disease parameters. To study the direct effects of TNF- α blockade on monocytic Fc γ R expression levels, monocytes were isolated and cultured with anti-TNF- α mAb. The effects were compared with those induced by TNF- α .

Results

Two weeks after the start of anti-TNF- α mAb therapy, monocytic Fc γ RI expression levels were decreased, whereas Fc γ RIIa and IIIa expression levels were unchanged. At 14 weeks, 8 weeks after the last gift of anti-TNF- α mAb, Fc γ RI expression levels returned to baseline levels. Fc γ RIIa and IIIa expression levels remained unchanged. The change in Fc γ RI correlated with changes in CRP and ESR levels. In vitro, anti-TNF- α mAb treatment did not alter expression of Fc γ RI on monocytes, but increased Fc γ RIIa and IIIa. TNF- α down-regulated all activating Fc γ Rs, mainly Fc γ RIIa and IIIa, but also the inhibitory Fc γ RIIb.

Conclusion

Anti-TNF- α mAb treatment of RA patients is accompanied by down-regulation of Fc γ RI expression levels on monocytes. This is likely an indirect effect of TNF- α blockade on disease activity, since in vitro anti-TNF- α mAb does not directly change Fc γ RI expression on monocytes. In contrast, TNF- α down-regulated all activating Fc γ Rs. Thus, blocking TNF- α may relieve the negative feedback mechanism of TNF- α as down-regulator of Fc γ Rs. Strategies to reduce activating Fc γ Rs may have additional value in the treatment of RA patients with TNF- α blockade by diminishing immune complex-mediated activation of monocytes/macrophages.

Key words

Rheumatoid arthritis, monocytes, Fc γ receptors, anti-TNF.

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Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disease, characterized by chronic synovitis, resulting in joint destruction. RA is also associated with systemic features like anemia, fever and rheumatoid nodules. Monocytes/macrophages play an important role in the pro-inflammatory response of RA. Activated monocytes/macrophages produce pro-inflammatory and tissue destructive mediators like interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α) and matrix metalloproteinases (MMPs), which contribute to chronic inflammation and bone and cartilage destruction (1-3). In RA patients immune complexes have been detected in serum, synovial fluid, synovium and cartilage. Various autoantibodies to joint-specific and ubiquitous antigens may be involved in RA. IgG-containing immune complexes, are able to activate monocytes/macrophages by binding to IgG Fc receptors (FcγRs) (4, 5). FcγRs bind the Fc domain of immunoglobulin G, which initiates a diversity of cellular functions such as phagocytosis, antigen presentation, antibody-dependent cellular cytotoxicity and the release of proinflammatory and tissue destructive mediators (6-8). Therefore throughout the body, but in particular in the joints, these immune complexes can trigger FcγR-mediated inflammation.

Three classes of FcγRs are currently recognized on human leukocytes: FcγRI, II and III, with the isoforms IIa and IIb, and IIIa and IIIb. All FcγRs vary in structure, cellular distribution, ligand-binding affinity and function (6-9). On human monocytes/macrophages FcγRI, IIa and IIb are constitutively expressed, whereas FcγRIIIa is only present on a small percentage of circulating monocytes (~10%) (10). FcγRIIIb is absent on monocytes and only present on polymorphonuclear cells. Functionally, two opposing types of FcγRs are characterized. FcγRI, IIa and IIIa are activating FcγRs, containing an intracellular tyrosine based activation motif (ITAM), which is activated upon ligation of the receptors. FcγRIIb is the only inhibitory FcγR, containing an intracellular tyrosine

based inhibitory motif (ITIM). Ligation of this receptor inhibits the activation of ITAM-containing receptors, via their intracellular motifs (11, 12). The balance between activating and inhibitory receptors will finally determine the extent of monocyte/macrophage activation. Modulation of FcγR expression levels may influence immune complex-mediated activation of monocytes/macrophages and consequently their pro-inflammatory responses.

Proinflammatory (*e.g.*, IFNγ and TNF-α) and anti-inflammatory cytokines (IL-4, IL-10, IL-13) which play an important role in the regulation of inflammation and tissue destruction in RA, influence FcγR expression levels (13-16). In RA patients with active disease, peripheral blood monocytes and synovial fluid macrophages display increased expression levels of activating FcγRs (17-19). FcγRIIb expression levels on monocytes in RA patients are similar to healthy controls (20). The altered balance of activating and inhibitory FcγRs in RA is suggested to contribute to chronic synovitis (20).

Blockade of pro-inflammatory mediators produced by monocytes/macrophages has led to an era of new biological therapies. Anti-TNF-α monoclonal antibodies (infliximab, adalimumab) (21), soluble TNF-α receptor (etanercept) (22, 23) and IL-1 receptor antagonist (anakinra) (24) have proven therapeutic value. Although these regimens neutralize inflammatory products of monocytes/macrophages and reduce inflammation, activation of monocytes/macrophages upon FcγR cross-linking (by immune complexes) may not be prevented. In this study we investigated the effect of blocking TNF-α by anti-TNF-α monoclonal antibody treatment (infliximab) on expression levels of activating FcγRs on monocytes, related to clinical parameters. For that reason, we performed a longitudinal study examining RA patients after the start of anti-TNF-α therapy. In addition, the direct effect of anti-TNF-α monoclonal antibodies on activating and inhibitory FcγR expression levels was tested *in vitro*.

Competing interests: none declared.

Materials and methods

Patients

Twelve RA patients (8 females, 4 males), visiting our outpatient clinic were studied. RA was diagnosed according to the 1987 revised ACR criteria for RA (25). They fulfilled the following inclusion criteria: previous use of at least 2 disease modifying anti-rheumatic drugs (DMARDs), one of them had to be methotrexate (MTX). They were either intolerant or had inadequate clinical responses for therapy. DMARD therapy, given alone or in combination, was continued and was not changed 3 months prior to inclusion. All patients had ≥ 6 swollen joints, erythrocyte sedimentation rate (ESR) ≥ 28 mm/hr and a disease activity score (DAS28) ≥ 3.6 (26). The RA patients ranged in age from 27 to 78 years (mean \pm SD; 52 \pm 14). Ten patients were RF positive. Disease duration varied from 3 to 38 years (mean 15 \pm 12).

All patients started treatment with infliximab (Remicade, Centocor, Leiden, The Netherlands) 3 mg/kg intravenously. They received infliximab at baseline (week 0) and at 2, 6 and 14 weeks. MTX was sustained in 7 patients, 6 patients received oral glucocorticosteroids, 2 patients continued using leflunomide, 1 hydroxychloroquine and 1 auranofine. At baseline and 14 weeks upon therapy, the following clinical and laboratory parameters were assessed: tender and swollen joint scores, morning stiffness, well-being (Visual analogue scale (VAS)), ESR and C-reactive protein (CRP). ESR and CRP levels were measured at two weeks as well. Venous blood samples were drawn at baseline, 2 weeks and 14 weeks just before infliximab infusion for *ex vivo* analysis of Fc γ R expression levels on monocytes. The study was performed according to the medical ethical guidelines of UMC Utrecht Medical Ethical Committee. Each patient gave informed consent.

Isolation of cells

Venous blood was diluted 1:1 with RPMI 1640 (Gibco Invitrogen, Life Technologies, United Kingdom) supplemented with 1% penicilline, streptomycine sulfate, glutamine (PSG). Peripheral blood mononuclear cells (PBMC) were iso-

lated by density gradient centrifugation using Ficoll Paque (Pharmacia Biotech, Uppsala, Sweden).

For *in vitro* analysis of infliximab, monocytes were isolated from PBMC of healthy donors by depletion of non-monocytes. In short, T cells, NK cells, B cells, dendritic cells and basophils were depleted using a monocyte isolation kit (Miltenyi Biotec, Bergisch Gladbach, Germany), containing a cocktail of hapten-conjugated CD3, CD7, CD19, CD45RA, CD56, and anti-IgE antibodies. Anti-Hapten magnetic microbeads were added and the magnetically labeled cells were depleted by retaining them on a MACS LS⁺ column using a MidiMACS magnet (Myltenyi). The negative effluent, containing untouched monocytes, was checked for cell purity by flow cytometry and always exceeded 95%.

Antibodies

For analysis of Fc γ R expression levels by flow cytometry the following FITC-conjugated monoclonal antibodies were used: anti-Fc γ RI (CD64; 32.2), anti-Fc γ RIIa (CD32; IV.3) and anti-Fc γ RIII (CD16; 3G8) (all from Medarex, Annandale, NJ). The isoforms Fc γ RIIa and I Ib are extracellularly 92% identical, but differ intracellularly (27). The CD32 monoclonal antibody IV.3 is directed against Fc γ RIIa and does not stain Fc γ RIIb (28). Discrimination between Fc γ RIIIa and Fc γ RIIIb is not possible using the CD16 monoclonal antibody 3G8. Fc γ RIIIb, however, is solely expressed on polymorphonuclear cells, which are not present in the mononuclear cell fraction used in our study. Other monoclonal antibodies used were: PE-conjugated CD14 (T μ k4, DAKO, Denmark), CD40 (5C3), CD80 (L307.4), CD86 (IT2.2) (all BD, PharMingen, San Diego, CA). FITC-conjugated HLAI (T μ 39, BD PharMingen) and an isotype matched control pair (FITC/PE) (Immunotech, Marseille, France).

FACS analysis

PBMC or monocytes were incubated for 30 minutes at 4°C with monoclonal antibodies, then washed in phosphate-buffered saline (PBS) containing 0.1%

sodium-azide and resuspended in the same buffer now containing 2% paraformaldehyde. Flow cytometry was performed on a FACScan (Becton Dickinson) and analyzed using CellQuest software. Fluorescence over time was checked and adjusted, using calibration beads (BD Immunocytometry Systems, San Jose, CA) as standards. For analysis of monocytes, gates were set around viable monocytes, based on their forward/sideward light scatter pattern and CD14 expression. Fc γ RI and I Ia are constitutively expressed on monocytes, whereas Fc γ RIIIa is only present on a unique subpopulation of monocytes. Fc γ RIIIa was therefore determined as the percentage Fc γ RIIIa⁺ CD14⁺ monocytes. With respect to Fc γ R expression levels, Fc γ RI, I Ia and I IIIa were determined as geometrical mean fluorescence intensity on CD14⁺ cells.

Monocytes incubated with infliximab *in vitro*

Freshly isolated untouched monocytes of healthy controls (n = 8) were cultured in the presence or absence of TNF- α (5 ng/ml, BD Pharmingen, San Diego, CA) or anti-TNF- α mAb (Remicade, Centocor, Leiden, The Netherlands) 5 μ g/ml for 2 days (29). Monocytes were cultured in flat bottom 24 wells microtiter plates at a concentration of 5 \times 10⁵ cells per well in a total volume of 1 ml RPMI (Gibco Invitrogen, Life Technologies, United Kingdom) supplemented with 1% penicilline, streptomycine sulfate, glutamine (PSG) and 10% human heat inactivated pooled AB⁺ serum (Red Cross Blood Transfusion Centre, Utrecht, The Netherlands). In addition to detect Fc γ RIIb on monocytes Fc γ RIIIa-H/H131 homozygous donors were selected.

To detect the inhibitory Fc γ RIIb on monocytes an antibody was used that recognizes a specific polymorphic site of Fc γ RII (described in detail in reference 20). This polymorphism is found in Fc γ RIIIa and is characterized by a variation at amino acid position 131, expressing either arginine (R131) or histidine (H131). Fc γ RIIb at this position always expresses an arginine (R131). In Fc γ RIIIa-H/H131 homozygous individuals Fc γ RIIIa only

contains a histidine at position 131, discriminating the activating FcγRIIIa from the inhibitory FcγRIIb that always contains an arginine. Thus to detect the FcγRIIb on monocytes we used a previously described monoclonal antibody (41H16-FITC) that specifically recognizes the arginine-containing site of FcγRIIb in FcγRIIIa-H/H131 homozygous donors.

Following culture, monocytes were harvested on ice and analyzed by flow cytometry for expression of FcγRI, IIa and IIIa as described above. To test the selectivity of FcγR regulation, the expression of CD40, CD80, CD86 and HLAII was also determined. Viability of monocytes was checked with Trypan Blue solution 0.4% (Sigma-Aldrich, Irvine, United Kingdom) and always exceeded 95%.

Statistical analysis

The Wilcoxon-signed ranks test was used to assess the significance of changes in FcγR expression levels and clinical parameters before and after the start of anti-TNF-α therapy. Correlations of changes in FcγRs and clinical parameters were evaluated with Spearman correlation analysis. The parametric paired samples *t*-test was used to compare FcγR expression levels of paired blood samples cultured with or without TNF-α or anti-TNF-α. A value of $p \leq 0.05$ was considered statistically significant.

Results

Downregulation of FcγRI expression levels on peripheral blood monocytes upon anti-TNFα treatment

FcγRI expression levels on peripheral blood monocytes were decreased two weeks after the first gift of anti-TNF-α mAb (representative staining Fig. 1A, individual patients Fig. 2A). The average \pm sd of FcγRI expression decreased from $MFI 55.8 \pm 10$ at baseline to $MFI 45.7 \pm 12$ at 2 weeks ($p = 0.02$). After 14 weeks, FcγRI expression levels were significantly higher compared to two weeks ($MFI 55.7 \pm 10$, $p = 0.01$), while similar to baseline levels (Fig. 2A). Expression levels of FcγRIIIa, FcγRIIIa were not significantly changed after 2 or 14 weeks when compared to

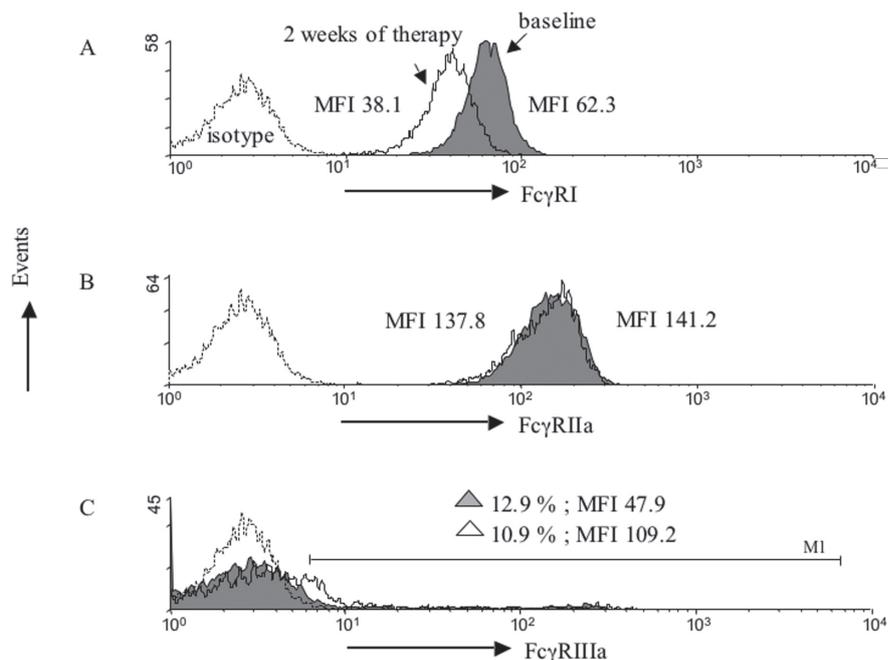


Fig. 1. Representative histograms of *ex vivo* expression levels of activating FcγRs on peripheral blood monocytes at baseline and 2 weeks after starting anti-TNF-α therapy. PBMC were stained for FcγRI (CD64), FcγRIIIa (CD32) or FcγRIIIa (CD16) together with CD14 (monocytes). The mean fluorescence intensity (MFI) of FcγRI (A), IIa (B) and IIIa (C) on CD14⁺ cells are given. The percentage FcγRIIIa⁺CD14⁺ cells, indicated by the marker line M1 that was set on basis of the isotype control, is also given (C). The filled histograms show levels at baseline, while the open histograms represent FcγR expression levels after 2 weeks of anti-TNF-α therapy. An isotype-matched control is depicted as a dotted line in all panels. The MFI is indicated for FcγR expression levels before and two weeks after treatment with anti-TNF-α therapy.

baseline levels (Representative FACS analyses Fig. 1B,C, individual patients Fig. 2B,C; MFI FcγRIIIa: baseline 143.0 ± 35 ; 2 weeks 136.6 ± 24 ; 14 weeks 159 ± 54 ; MFI FcγRIIIa: baseline 65.3 ± 27 ; 2 weeks 68.0 ± 25 ; 14 weeks 69.8 ± 31). In addition, the percentage of FcγRIIIa⁺ monocytes was not significantly changed by anti-TNF-α therapy (%FcγRIIIa⁺ monocytes: baseline 19 ± 9 ; 2 weeks 16 ± 8 ; 14 weeks 20 ± 14 ; Fig. 2D). The percentage monocytes (CD14⁺ cells) also did not change upon anti-TNF-α therapy (data not shown).

Relationship of FcγRs and clinical responses

Two weeks after the first gift of anti-TNF-α monoclonal antibodies, levels of CRP and ESR decreased significantly (CRP at baseline mean \pm sd; 24 ± 17 mg/l, at 2 weeks 10 ± 11 , $p = 0.003$; ESR at baseline 65 ± 28 mm/hr, at 2 weeks 36 ± 26 , $p = 0.025$). At 14 weeks, 8 weeks after the third gift, before a new gift of anti-TNF-α mAb, CRP and ESR levels returned to baseline levels (CRP;

20 ± 20 mg/l, $p = 0.046$) (ESR; 53 ± 36 mm/hr, ns) (Fig. 3). Clinical parameters like tender and swollen joint scores, morning stiffness and well-being (VAS) had similar responses from baseline to 14 weeks after the start of infliximab. Changes in FcγRI expression levels correlated with changes in CRP ($\rho = 0.451$; $p = 0.02$) and ESR levels ($\rho = 0.486$; $p = 0.04$). No significant correlations between changes in FcγRIIIa and FcγRIIIa and disease parameters were found.

No change in FcγRI on monocytes upon in vitro exposure to anti-TNF-α TNF-γ (5 ng/ml) modulates monocyte FcγR expression by down-regulating all activating FcγRs (FcγRI: $-14\% \pm 6$, $p < 0.01$; FcγRIIIa: $-29\% \pm 11$, $p < 0.01$; FcγRIIIa: $-22\% \pm 17$, $p < 0.01$) (Fig. 4A). To determine the effect of TNF-α blockade on the expression levels of FcγRs, isolated monocytes of healthy controls were cultured in the presence of infliximab (5 μg/ml). After two days, monocytes cultured in the presence of infliximab displayed higher expres-

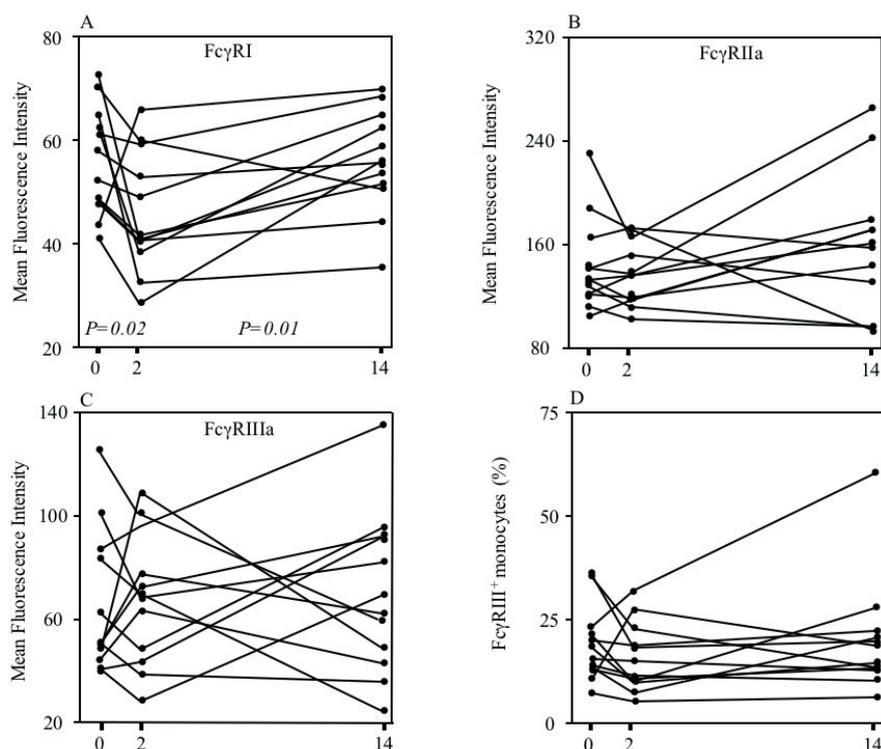
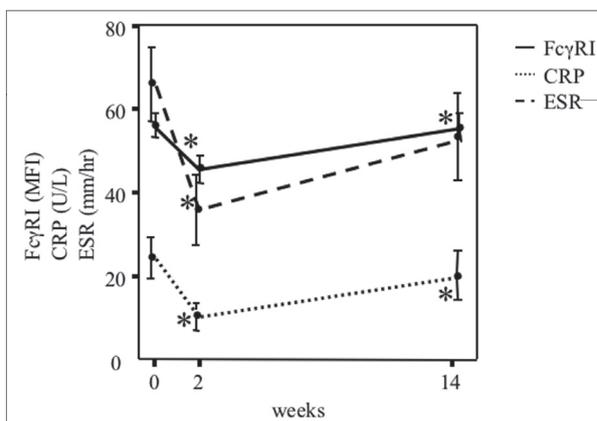


Fig. 2. Expression levels of activating FcγRs on peripheral blood monocytes of individual RA patients at baseline, 2 weeks and 14 weeks after starting anti-TNF-α therapy. PBMC of RA patients ($n = 12$) were analyzed by flow cytometry for FcγRI, IIa and IIIa expression levels on CD14⁺ cells. The mean fluorescence intensities (MFI) are given of FcγRI (A) (mean \pm SD): baseline: 55.8 ± 10 ; 2 weeks: 45.7 ± 12 ; 14 weeks: 55.7 ± 10 , FcγRIIa (B) baseline: 143 ± 35 ; 2 weeks: 136.6 ± 24 ; 14 weeks: 159 ± 54 and FcγRIIIa (C) baseline: 65.3 ± 27 ; 2 weeks: 68 ± 25 ; 14 weeks: 69.8 ± 31 on CD14⁺ cells. (D) The percentage FcγRIIIa⁺CD14⁺ cells is also shown: baseline: 19 ± 9 ; 2 weeks: 16 ± 8 ; 14 weeks: 20 ± 14 .

Fig. 3. Effect of anti-TNF-α therapy on markers of inflammation (disease activity) and monocytic FcγRI expression levels. Patients received an anti-TNF-α mAb infusion at baseline, 2, 6 and 14 weeks. Mean levels (\pm sem) of FcγRI, CRP and ESR at baseline, 2 and 14 weeks are given. FcγRI: baseline: 55.8 ± 3 ; 2 weeks: 45.7 ± 3 ; 14 weeks: 55.7 ± 3 ; CRP: baseline: 24 ± 5 ; 2 weeks: 10 ± 3 ; 14 weeks: 20 ± 6 ; ESR: baseline: 65 ± 9 ; 2 weeks: 36 ± 8 ; 14 weeks: 53 ± 10 . Asterisks indicate statistical significant differences of $p < 0.05$. Changes in FcγRI expression levels on monocytes from RA patients correlate with changes in CRP ($\rho = 0.451$; $p = 0.02$) and ESR levels ($\rho = 0.486$; $p = 0.04$).



sion levels of FcγRIIIa ($+32.5\% \pm 34$, $p < 0.01$) and FcγRIIIa ($+34.5\% \pm 41$, $p = 0.04$) compared to control conditions, whereas FcγRI expression levels were similar (Fig. 4B). In addition, in FcγRIIIa-131 histidine homozygous donors we stained FcγRIIIb on monocytes with an antibody that specifically recognizes the 131 arginine site, which is

at all times expressed by FcγRIIIb (Fig. 4C). TNF-α tested *in vitro* down-regulated FcγRIIIb (mean \pm SD; $-28\% \pm 16$, $p < 0.05$), while anti-TNF-α mAb *in vitro* slightly up-regulated FcγRIIIb ($+14\% \pm 7$) on the monocytes of these healthy donors.

To test the selectivity of anti-TNF-α on FcγRs, other surface markers on mono-

cytes were determined as well. The expression levels of CD40 were reduced by anti-TNF-α treatment compared to control conditions ($-20\% \pm 9$, $p < 0.01$), whereas CD86 ($+86.5\% \pm 41$, $p < 0.01$), and HLA class II ($+28.3\% \pm 33$, $p = 0.03$) expression levels were higher in the presence of infliximab. CD80 and CD14 expression levels on monocytes were not significantly changed by culture in the presence of infliximab.

Discussion

Treatment of RA patients with anti-TNF-α mAb showed a down-regulation of FcγRI expression levels two weeks after the first gift, in contrast to unchanged FcγRIIa and IIIa expression levels. Changes in FcγRI expression levels were associated with changes in markers of inflammation (CRP and ESR).

The decline in FcγRI expression levels upon anti-TNF-α therapy after 2 weeks may result from a direct effect of TNF-α blockade or indirectly be the result of clinical improvement. Our *in vitro* data suggest that the direct effect of TNF-α blockade on FcγRI expression by monocytes may be minimal. FcγRI expression levels on monocytes were not significantly changed upon TNF-α blockade *in vitro*, whereas FcγRIIa and FcγRIIIa expression levels were significantly increased. In addition, TNF-α treatment *in vitro* slightly down-regulated FcγRI and to a greater extent down-regulated FcγRIIa and FcγRIIIa expression levels on monocytes. Together the direct *in vitro* effects of anti-TNF-α and TNF-α can not explain the decrease of FcγRI and unchanged FcγRIIa and FcγRIIIa expression levels found upon anti-TNF-α therapy. Therefore, reduction of FcγRI upon anti-TNF-α therapy is likely the result of clinical improvement. The fact that FcγRI expression levels return to baseline levels at 14 weeks, 8 weeks after the last gift of infliximab, is in line with this suggestion. At this time point, many patients experience loss of efficacy of the treatment (data not shown), accompanied by a raise in CRP and ESR levels.

The changes in FcγRI expression levels were associated with the changes in both CRP and ESR. A correlation

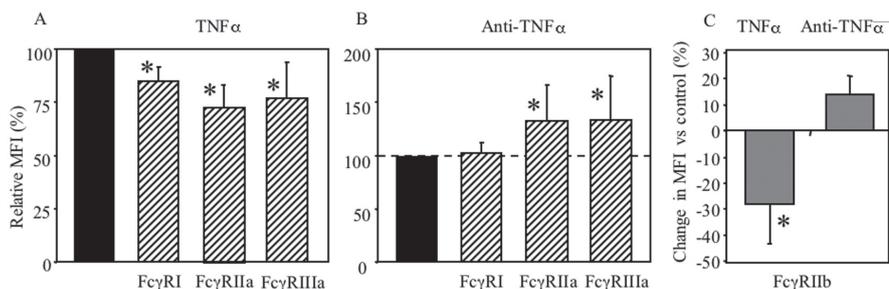


Fig. 4. Monocytes of healthy controls ($n = 8$) were cultured for 2 days in the absence or presence of TNF- α (5ng/ml) (A) or anti-TNF- α mAb (infliximab, 5 μ g/ml) (B), where after Fc γ R expression levels were determined by flow cytometry. To detect Fc γ RIIb, 131 histidine homozygous donors were used, staining Fc γ RIIb with 131 arginine recognizing antibody 41H16 (C). The relative mean fluorescence intensity (MFI) of Fc γ RI (CD64), Fc γ RIIa (CD32) and Fc γ RIIIa (CD16) on monocytes cultured in the presence of TNF- α or anti-TNF- α mAb are given (shaded bars) compared to cultures without (100%, black bar). Asterisks indicate statistical significant differences between cultures with and without TNF- α or with and without anti-TNF- α mAb of $p < 0.05$.

between increased CRP and increased Fc γ RI expression levels on monocytes in RA patients has been demonstrated previously upon treatment with methotrexate or glucocorticoids (30, 31). The decreased levels of CRP may be a direct result from blocking TNF- α that down-regulates the production of IL-1 β and IL-6. TNF- α , IL-1 β and IL-6 can all induce hepatic production of CRP (32, 33). On the other hand, decreased immune complex-stimulation via Fc γ RI, indirectly as a consequence of initial disease improvement, can lead to a further reduction of cytokine production as well and thereby prevent induction of CRP. The importance of Fc γ RI is also underlined in a murine experimental arthritis model, being the dominant Fc γ R to play a role in cartilage destruction (34, 35). It seems likely that the decreased levels of Fc γ RI can both be caused by and contribute to a reduction in disease activity upon anti-TNF- α therapy.

Although Fc γ RIIa and IIIa expression levels have been shown to be increased on monocytes of RA patients (17-19, 19), no reduction upon anti-TNF- α therapy was found. It may be speculated that down-regulation of Fc γ RIIa and Fc γ RIIIa expression levels as a consequence of disease improvement is counteracted by the direct up-regulatory effect of anti-TNF- α mAb on monocytic Fc γ R expression, which we demonstrated *in vitro*.

In RA, high expression levels of activating Fc γ Rs, mainly Fc γ RIIa and IIIa, have been held responsible for the

production of TNF- α upon immune complex stimulation (36, 37). A combination of blocking pro-inflammatory products of monocytes/macrophages and down-modulating activating Fc γ R expression levels to prevent monocyte/macrophage activation may therefore have therapeutic value. Recently we have shown that the disease modifying effect of MTX (*ex vivo*) is accompanied by a down regulation of activating Fc γ RI and IIa (30). Since this effect was also seen *in vitro* on isolated monocytes this could well be a direct effect of MTX on monocytes. In line with this suggestion co-therapy of anti-TNF- α mAb with MTX has shown additive effects on clinical improvement (38, 39). Direct down-regulation of activating Fc γ Rs by MTX might contribute to this additional benefit. In the present study 58% of the RA patients used MTX. However, the study population was too small to investigate the beneficial effect of MTX *plus* anti-TNF- α on Fc γ R expression and clinical outcome. Furthermore, MTX was used for prolonged periods (> 3 months) prior to anti-TNF- α therapy, making an evaluation of the effect of MTX on Fc γ R expression levels in this population impossible. Other possible strategies to down-regulate activating Fc γ Rs and prevent immune complex-triggered TNF- α production are IL-4 and the combination of IL-4 plus IL-10. Recently we have shown that IL-4 and IL-4 + IL-10 can alter the balance of activating and inhibitory Fc γ Rs by inhibition of activating Fc γ Rs (IL-4

or induction of the inhibitory Fc γ RIIb (IL-4 + IL-10) (20).

The balance between activating and inhibitory Fc γ Rs importantly determines the height of effector cells and thereby inflammatory responses (8, 9, 20). In the present study we described the *in vivo* effects of anti-TNF- α mAb on activating Fc γ Rs. In this clinical study for technical reasons we were not able to measure the inhibitory Fc γ RIIb. However, using a recently developed new method to detect Fc γ RIIb on monocytes (20), we here demonstrate that TNF- α *in vitro* down-regulates Fc γ RIIb, while anti-TNF- α mAb *in vitro* slightly up-regulates Fc γ RIIb on monocytes. Since the direct effects of TNF- α and anti-TNF- α on Fc γ RIIb seem to parallel the effects on activating Fc γ Rs, these data suggest that the balance of activating and inhibitory Fc γ Rs on monocytes is not directly changed by anti-TNF- α mAb.

Anti-TNF- α monoclonal antibodies (mAb) block an important mediator of the pro-inflammatory and tissue destructive immune response produced by activated monocytes/macrophages in RA patients. We demonstrated that treatment with anti-TNF- α mAb is accompanied by a rapid down-regulation of Fc γ RI, which may strongly contribute to the success of anti-TNF- α therapy by diminishing immune complex-mediated activation of monocytes/macrophages. However, monocytes tested *in vitro* with anti-TNF- α mAb expressed higher Fc γ RIIa and Fc γ RIIIa and immune complex-mediated activation of monocytes/macrophages may therefore not be prevented. Strategies aimed at down-regulation of all activating Fc γ Rs (Fc γ RIIa and Fc γ RIIIa) on monocytes and changing the balance of activating and inhibitory Fc γ Rs in favour of the inhibitory Fc γ RIIb, may therefore have additional value in the treatment of RA, either alone or in combination of TNF- α blockade.

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