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

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Abstract

Objectives

To study the effect of anti-TNF-α therapy on activating IgG Fc receptor (FcγR) 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, FcγR 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 FcγR 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 FcγRI expression levels were decreased, whereas FcγRIIa and IIIa expression levels were unchanged. At 14 weeks, 8 weeks after the last gift of anti-TNF-α mAb, FcγRI expression levels returned to baseline levels. FcγRIIa and IIIa expression levels remained unchanged. The change in FcγRI correlated with changes in CRP and ESR levels. In vitro*, anti-TNF-α mAb treatment did not alter expression of FcγRI on monocytes, but increased FcγRIIa and IIIa. TNF-α down-regulated all activating FcγRs, mainly FcγRIIa and IIIa, but also the inhibitory FcγRIIb.*

Conclusion

Anti-TNF-α mAb treatment of RA patients is accompanied by down-regulation of FcγRI 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 FcγRI expression on monocytes. In contrast, TNF-α down-regulated all activating FcγRs. Thus, blocking TNF-α may relieve the negative feedback mechanism of TNF-α as down-regulator of FcγRs. Strategies to reduce activating FcγRs 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

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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 monocyt/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 regiments 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*.

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) ≥ 28mm/hr and a disease activity score (DAS28) ≥ 3.6 (26). The RA patients ranged in age from 27 to 78 years (mean ± SD; 52 ± 14). Ten patients were RF positive. Disease duration varied from 3 to 38 years (mean 15 ± 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 FcγR 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 isolated 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 (Mylteni). The negative effluent, containing untouched monocytes, was checked for cell purity by flow cytometry and always exceeded 95%.

Antibodies

For analysis of FcγR expression levels by flow cytometry the following FITC-conjugated monoclonal antibodies were used: anti-FcγRI (CD64; 32.2), anti-FcγRIIa (CD32; IV.3) and anti-FcγRIII (CD16; 3G8) (all from Medarex, Annandale, NJ). The isoforms FcγRIIa and IIb are extracellularly 92% identical, but differ intracellularly (27). The CD32 monoclonal antibody IV.3 is directed against FcγRIIa and does not stain FcγRIIb (28). Discrimination between FcγRIIIa and FcγRIIIb is not possible using the CD16 monoclonal antibody 3G8. FcγRIIIb, 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 (Tük4, DAKO, Denmark), CD40 (5C3), CD80 (L307.4), CD86 (IT2.2) (all BD, PharMingen, San Diego, CA). FITC-conjugated HLAII (Tü39, 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. FcγRI and IIa are constitutively expressed on monocytes, whereas FcγRIIIa is only present on a unique suppopulation of monocytes. FcγRIIIa was therefore determined as the percentage $FcγRIIIa^+$ $CD14^+$ monocytes.With respect to FcγR expression levels, FcγRI, IIa and 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 μg/ml for 2 days (29). Monocytes were cultured in flat bottom 24 wells microtiter plates at a concentration of $5x10^5$ 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 FcγRIIb on monocytes FcγRIIa-H/H131 homozygous donors were selected.

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

contains a histidine at position 131, discriminating the activating FcγRIIa 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 FcγRIIa-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 ± sd of FcγRI expression decreased from MFI 55.8 ± 10 at baseline to MFI 45.7 ± 12 at 2 weeks ($p = 0.02$). After 14 weeks, FcγRI expression levels were significantly higher compared to two weeks (MFI 55.7 ± 10, $p = 0.01$), while similar to baseline levels (Fig. 2A). Expression levels of FcγRIIa, FcγRIIIa were not significantly changed after 2 or 14 weeks when compared to baseline levels (Representative FACS analyses Fig. 1B,C, individual patients Fig.2B,C; MFI FcγRIIa: 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).

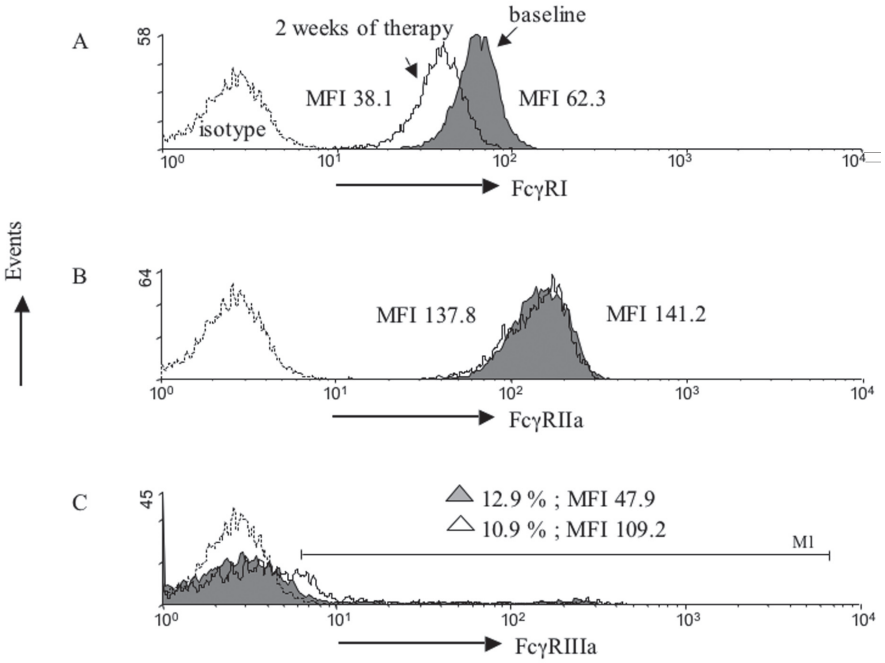

Fig. 1. Representative histograms of *ex vivo* expression levels of activating FcγRs on peripheral blood monocytes at baseline and 2 weeks after starting anti-TNF-α therapy. PBMC were stained for FcγRI (CD64), FcγRIIa (CD32) or FcγRIIIa (CD16) together with CD14 (monocytes). The mean fluorescence intensity (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.

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 ± 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 FcγRIIa 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 monocytic FcγR expression by down-regulating all activating FcγRs (FcγRI: –14% ± 6, $p < 0.01$; FcγRIIa: -29% ± 11, $p < 0.01$; FcγRIIIa: –22% ± 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

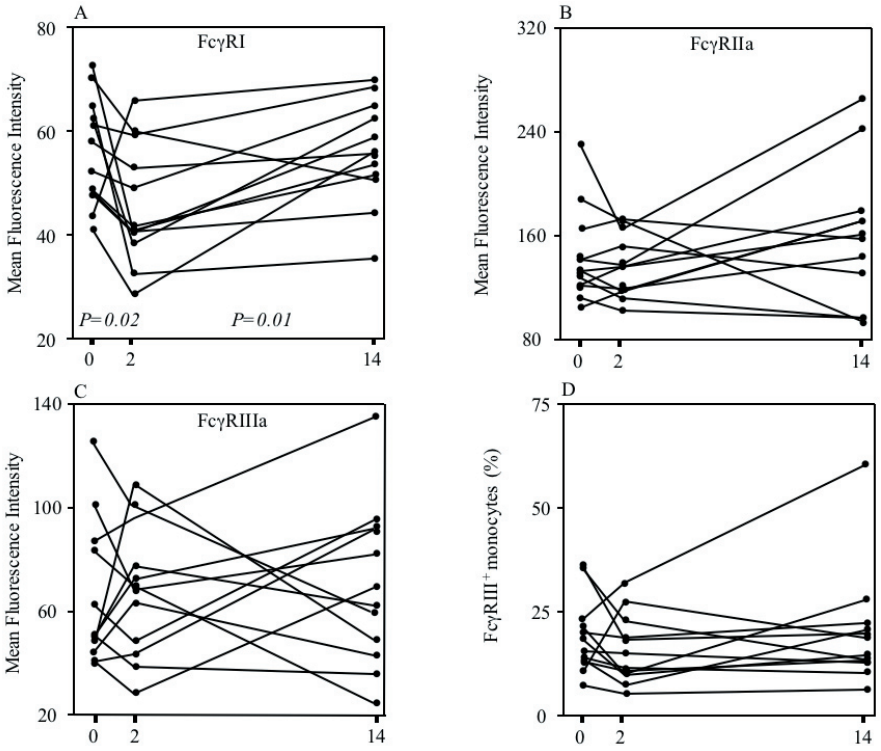

Fig. 2. Expression levels of activating FcγRs on peripheral blood monocytes of individual RA patients at baseline, 2 weeks and 14 weeks after starting anti- TNF-α therapy. PBMC of RA patients (n = 12) were analyzed by flow cytometry for FcγRI, IIa and IIIa expression levels on $CD14^+$ cells. The mean fluorescence intensities (MFI) are given of FcγRI (A) (mean ± 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.

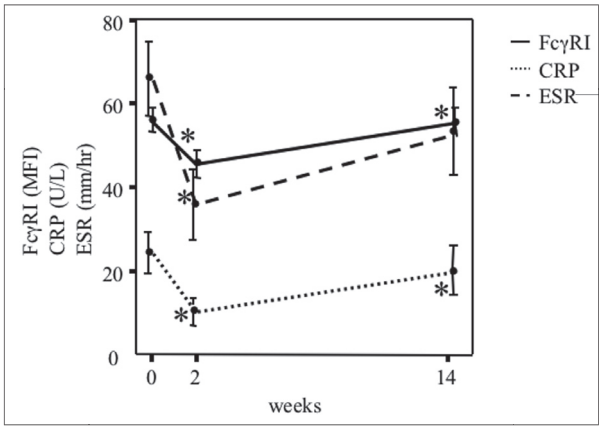

Fig. 3. Effect of anti-TNF-α therapy on markers of inflammation (disease activity) and monocytic FcγRI expression levels. Patients received an anti-TNF-α mAb infusion at baseline, 2, 6 and 14 weeks. Mean levels (± sem) of FcγRI, CRP and ESR at baseline, 2 and 14 weeks are given. FcγRI: baseline: 55.8 ± 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 ($\rho = 0.486$; $p = 0.04$).

expression levels of FcγRIIa (+32.5% ± 34 , $p < 0.01$) and FcγRIIIa (+34.5% ± 41, $p = 0.04$) compared to control conditions, whereas FcγRI expression levels were similar (Fig. 4B). In addition, in FcγRIIa-131 histidine homozygous donors we stained FcγRIIb on monocytes with an antibody that specifically recognizes the 131 arginine site, which is at all times expressed by FcγRIIb (Fig. 4C). TNF-α tested *in vitro* down-regulated FcγRIIb (mean ± SD; -28% ± 16, $p < 0.05$), while anti-TNF-α mAb *in vitro* slightly up-regulated FcγRIIb (+14% ±7) on the monocytes of these healthy donors.

To test the selectivity of anti-TNF-α on FcγRs, other surface markers on monocytes were determined as well. The expression levels of CD40 were reduced by anti-TNF-α treatment compared to control conditions (-20% ± 9, $p < 0.01$), whereas CD86 (+86.5% ± 41, $p < 0.01$), and HLA class II (+ 28.3% ± 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

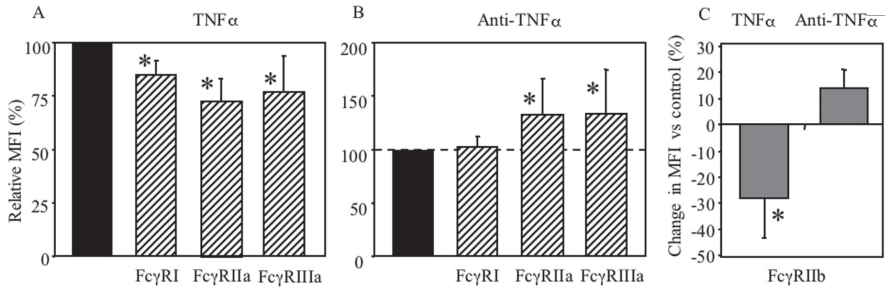

Fig. 4. Monocytes of healthy controls (n = 8) were cultured for 2 days in the absence or presence of TNF-α (5ng/ml) (A) or anti-TNF-α mAb (infliximab, 5μg/ml) (B), where after FcγR expression levels were determined by flow cytometry. To detect FcγRIIb, 131 histidine homozygous donors were used, staining FcγRIIb with 131arginine recognizing antibody 41H16 (C). The relative mean fluorescence intensity (MFI) of FcγRI (CD64), FcγRIIa (CD32) and FcγRIIIa (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 FcγRI 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-1β and IL-6. TNF-α, IL-1β and IL-6 can all induce hepatic production of CRP (32, 33). On the other hand, decreased immune complex-stimulation via FcγRI, 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 FcγRI is also underlined in a murine experimental arthritis model, being the dominant FcγR to play a role in cartilage destruction (34, 35). It seems likely that the decreased levels of FcγRI can both be caused by and contribute to a reduction in disease activity upon anti-TNF-α therapy.

Although FcγRIIa 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 FcγRIIa and FcγRIIIa expression levels as a consequence of disease improvement is counteracted by the direct up-regulatory effect of anti-TNF-α mAb on monocytic FcγR expression, which we demonstrated *in vitro*.

In RA, high expression levels of activating FcγRs, mainly FcγRIIa 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 FcγR 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 FcγRI 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 FcγRs 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 FcγR 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 FcγR expression levels in this population impossible. Other possible strategies to down-regulate activating FcγRs 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 FcγRs by inhibition of activating FcγRs (IL-4) or induction of the inhibitory FcγRIIb (20).

The balance between activating and inhibitory FcγRs 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 FcγRs. In this study for technical reasons we were not able to measure the inhibitory FcγRIIb. However, using a recently developed new method to detect FcγRIIb on monocytes (20), we here demonstrate that TNF-α *in vitro* down-regulates FcγRIIb, while anti-TNF-α mAb *in vitro* slightly up-regulates FcγRIIb on monocytes. Since the direct effects of TNF-α and anti-TNF-α on FcγRIIb seem to parallel the effects on activating FcγRs, these data suggest that the balance of activating and inhibitory FcγRs 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 FcγRI, 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 FcγRIIa and FcγRIIIa and immune complex-mediated activation of monocytes/macrophages may therefore not be prevented. Strategies aimed at down-regulation of all activating FcγRs (FcγRIIa and FcγRIIIa) on monocytes and changing the balance of activating and inhibitory FcγRs in favour of the inhibitory FcγRIIb, may therefore have additional value in the treatment of RA, either alone or in combination of TNF-α blockade.

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