Influence of therapy with chimeric monoclonal
tumour necrosis factor-α antibodies on
intracellular cytokine profiles of T lymphocytes
and monocytes in rheumatoid arthritis patients

A. J. Schuerwegh, J. F. Van Offel, W. J. Stevens, C. H. Bridts and
L. S. De Clerck

Introduction. It has been shown that T lymphocytes and monocytes/macrophages,
producing pro-inflammatory cytokines, play a pivotal role in the pathophysiology
of rheumatoid arthritis (RA). In recent placebo-controlled double-blind randomized studies, chimeric (human-mouse) tumour necrosis factor-α (TNFα)
antibodies (cA2) proved to be very effective in improving clinical disease activity
and reducing inflammatory parameters in RA.

Objective. To investigate whether anti-TNFα therapy influences the in vitro
intracellular cytokine production in peripheral blood monocytes and T lymphocytes of RA patients after one single (24 h) and multiple intravenous infusions
(6 months).

Methods. An intracellular flow cytometric technique was applied to measure
interleukin 1β (IL-1β), IL-6, TNFα, IL-10 and IL-12 in monocytes and IL-2, IL-4
and interferon-γ in T lymphocytes of 15 patients, before, after 24 h and after
6 months of therapy with monoclonal chimeric anti-TNFα antibodies (3 mg kg
bimonthly i.v.). All patients were on stable therapy with methotrexate (15–
20 mg week i.m.). Cytokine content in monocytes was measured directly after
blood sampling (basal levels), after 8 h of culture (spontaneous production) and
after 8 h of stimulation with lipopolysaccharides (LPS-stimulated production).

Results. Basal levels and production (after 8 h) of IL-1β, IL-6 and TNFα were
significantly decreased 24 h after the first administration of anti-TNFα (for IL-1β
P < 0.01; for IL-6 P < 0.01; for TNF α P < 0.003) and after 6 months of therapy
(for IL-1β P < 0.02; for IL-6 P < 0.03; for TNFα P < 0.001). For IL-12, basal
levels were significantly decreased 24 h and 6 months after the start of therapy
with anti-TNFα antibodies (P = 0.0001; P = 0.003, respectively). In contrast, IL-10
production increased significantly after 24 h and after 6 months (P = 0.02;
P = 0.01). The \( \frac{T_{H2}}{T_{H1}} \) cytokine ratio in CD4+ T cells was significantly increased
after 24 h and after 6 months of anti-TNFα therapy (P = 0.003; P = 0.0007).

Conclusion. Anti-TNFα therapy might down-regulate the monocytic capacity to
produce pro-inflammatory cytokines and induces a shift to a more pronounced
anti-inflammatory T\( \text{H}_2 \) cytokine production.

Key words: Tumour necrosis factor-α, Anti-TNFα therapy, RA, Cytokine.
Previous investigations have revealed the crucial role of inflammatory cells (T lymphocytes, B cells, monocytes, macrophages) and pro-inflammatory cytokines [interleukin 1β (IL-1β), IL-6, tumour necrosis factor-α (TNFα), interferon-γ, IL-1x] in the pathogenesis of rheumatoid arthritis (RA) [1–3]. The rationale for blockade of TNFα was based upon several reports that demonstrated the particular importance of TNFα: high concentrations of TNFα were observed in synovial fluid, synoviocytes and synovial macrophages of patients with RA [4, 5]. Injection of TNFα in animals resulted in synovitis with infiltration of lymphocytes, monocytes and neutrophils in the articular cavity [6]. In addition, a relationship between synovial TNFα expression and disease activity in RA patients was observed [7]. In collagen-induced arthritis in mice, treatment with monoclonal antibodies to TNFα was able to diminish the production of IL-1 and granulocyte–macrophage colony-stimulating factor (GM-CSF) by synovial cells in vitro and to ameliorate synovitis and joint destruction [8–10]. These findings led to the conclusion that TNFα is an important therapeutic target in rheumatoid arthritis: in recent, placebo-controlled, double-blind, randomized studies, anti-TNFα therapy proved to be very effective in improving clinical and laboratory disease activity and reducing inflammatory parameters [11–18].

To further investigate the possible mechanisms responsible for the clinical efficacy of anti-TNFα therapy, we studied the short-term (24 h) and long-term (6 months) effects of anti-TNFα therapy on in vitro intracellular cytokine production in T lymphocytes and monocytes.

**Methods and patients**

**Study population**

Fifteen patients with active rheumatoid arthritis (RA), fulfilling the diagnostic criteria of the American College of Rheumatology for the classification of rheumatoid arthritis [19], were evaluated before, after 24 h and after 6 months (just before administering the sixth infusion) of therapy with monoclonal chimeric anti-TNFα antibodies (3 mg/kg i.v. at week 0, week 2, week 6, week 14, week 22, week 30) in combination with stable doses of methotrexate (12.5–20 mg/week i.m.) and stable low-dose corticosteroids (5–10 mg/day) or non-steroidal anti-inflammatory drugs (NSAIDs) (Table 1). The disease duration of the RA patients was 47 (23–279) months [median (range)]. Active disease was considered if the following criteria were present: morning stiffness lasting for more than 20 min, presence of at least six painful joints and four swollen joints and an erythrocyte sedimentation rate (ESR) of more than 28 mm/h or a C-reactive protein (CRP) of at least 1 mg/dl. The clinical response at month 6 was performed by a blinded assessor and defined according to the American College of Rheumatology definitions of ACR 20, ACR 50 and ACR 70 [20]. A placebo-controlled, double-blind study was ethically not acceptable since the efficacy of anti-TNFα therapy is already well established in placebo-controlled, double-blind studies [11, 14]. All patients gave informed consent for the study.

**Analysis of lymphocyte subsets**

An aliquot of 100 μl of peripheral blood was incubated for 15 min in the dark at 4°C with 10 μl of multi-colour monoclonal antibody panels (CD3-PerCP + CD4-FITC + CD8-PE; CD45-PerCP + CD3-FITC + CD19-PE; BD Biosciences, Erembodegem, Belgium). Subsequently, the remaining red blood cells were lysed with Facslysis (BD Biosciences) for 10 min at room temperature. Cells were fixed and pelleted at 400 g for 10 min. Afterwards, the cells were resuspended in phosphate-buffered saline (PBS) and analysed by FACScan flow cytometry (BD) within 24 h.

**Intracellular cytokine analysis in lymphocytes**

Cytokine-producing T lymphocytes were determined as previously described [21]. Briefly, peripheral whole blood cultures were incubated for 6 h at 37°C and 5% CO₂ humidified atmosphere in the presence of 50 ng/ml phorbol-12-myristate-13-acetate (PMA, Sigma, St Louis, MO, USA), 1 μM Ionomycin (Sigma) and 1 μg/ml brefeldin A (Sigma) or brefeldin A alone. After stimulation, 100 μl of cells were stained with CD3-FITC (BD) and CD3-PerCP (BD). Cells were lysed with Facslysis (BD) and permeabilized in 0.3% saponin in PBS. Cells were stained for 30 min with monoclonal anti-cytokine antibodies labelled with phycoerythrin (PE) (anti-Hu IL-2-PE, anti-Hu IL-4-PE, anti-interferon-PE; BD). Twenty thousand cells were measured on a FACScan (BD) flow cytometer and analysed with WinMDI 2.8 software. Analysis gates were set on lymphocytes according to forward and sideward scatter properties. CD4+ T lymphocytes were defined and gated out as CD3+CD8- and CD8+ T cells as CD3+CD8+ using a compensated fluorescence plot. Within this gate, intracellular cytokine-producing cells were determined in histogram mode (PE emission). Markers were set on the 99th percentile using isotype-matched irrelevant antibodies (mouse IgG1 RPE, Serotec Ltd, Oxford, UK) as a reference. Results were expressed as the percentage of cytokine-producing cells. T helper (Th2/Th1) and T cytotoxic (Tc2/Tc1) ratios were calculated in the following manner: values for IL-4 (representing a Th2 cytokine)-positive CD4+ or CD8+ T lymphocytes were divided by values for interferon-γ or IL-2 (representing a Th1/Tc1 cytokine)-positive CD4+ or CD8+ T lymphocytes.

**Intracellular cytokine analysis in monocytes**

Intracellular cytokine production by monocytes was determined as previously described [22]. Cytokine content in monocytes was measured directly after blood sampling (basal level), after 8 h of culture (spontaneous production) and after 8 h of stimulation with lipopolysaccharides (LPS-stimulated production). Peripheral blood cultures were stimulated for 8 h with 1 μg/ml lipopolysaccharide Escherichia coli (LPS, Serotype 026:B6, Sigma) and 1 μg/ml brefeldin A (LPS-stimulated

<table>
<thead>
<tr>
<th>Table 1. Characteristics of RA patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr) 57 (35–76)</td>
</tr>
<tr>
<td>Female/male 13/2</td>
</tr>
<tr>
<td>Duration of disease (months) 47 (23–279)</td>
</tr>
<tr>
<td>Dose of methotrexate (mg/week) 15 (12.5–20)</td>
</tr>
<tr>
<td>Glucocorticoid therapy (%) 47 (7/15)</td>
</tr>
<tr>
<td>NSAID (%) 53 (8/15)</td>
</tr>
</tbody>
</table>

Results are expressed as median (range).
production), or brefeldin A alone (spontaneous production). After culture, or immediately after blood sampling (to evaluate basal cytokine levels in monocytes), 100 μl of whole blood was stained with CD14-FITC (BD), lysed with FacsLysis buffer (BD), fixed and made permeable. Cells were incubated with PE-labelled anti-cytokine antibodies against IL-1β, IL-6 and TNFα (BD).

Forty thousand events were measured on a FACScan flow cytometer and analysed with WinMDI software. Analysis gates were set on CD14+ cells according to fluorescein isothiocyanate (FITC) emission and side scatter. Within this gate, intracellular cytokine production was evaluated. Measurements were standardized using microspheres with different fluorescence intensities (DAKO FluoroSpheres, Code No K 0110 Glostrup, Denmark). Results were expressed as molecules of equivalent soluble fluorescein (MESF) units. Isotype-matched irrelevant antibodies (mouse IgG1 RPE, Serotec) were used as controls.

Statistics

Differences in numbers of T-cell subsets and cytokine production before and after therapy were calculated with Friedman’s test and Wilcoxon’s rank test. Correlations were assessed with Spearman’s rank correlation test.

Results

Disease activity

After 6 months, 14 patients showed reduced symptoms and signs of RA (Table 2), as judged by ACR 20 (20% of improvement); nine of 15 patients achieved ACR 50.

White blood cell counts

Therapy with anti-TNFα resulted in an increase in lymphocyte counts in the peripheral blood, 24 h after the first infusion (Table 3). The elevation of lymphocyte numbers was mainly due to an absolute increase of CD3+ T cells (P=0.0001) (Table 3). The absolute number of CD4+ T cells increased drastically after 24 h (P=0.0003), with no differences after 6 months (Table 3). Similarly, the number of CD8+ T cells increased after 24 h (P=0.0006). There was no change in numbers of peripheral blood B lymphocytes (CD19+). In addition, a rapid decrease of absolute numbers of monocytes (CD14+) was found (P=0.01) (Table 3).

Table 2. Clinical parameters in RA patients before (d0) and after 6 months of therapy (m6) with anti-TNFα

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Before therapy d0</th>
<th>After therapy m6</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESR (mm h)</td>
<td>43 (25–110)</td>
<td>18 (2–34)</td>
<td>0.04</td>
</tr>
<tr>
<td>CRP (mg dl)</td>
<td>2.6 (0.46–9.36)</td>
<td>&lt;0.35 (&lt;0.35–1.94)</td>
<td>0.03</td>
</tr>
<tr>
<td>Morning stiffness (min)</td>
<td>180 (60–240)</td>
<td>0 (0–180)</td>
<td>0.02</td>
</tr>
<tr>
<td>Ritchie index a</td>
<td>24 (9–38)</td>
<td>2 (0–13)</td>
<td>0.0007</td>
</tr>
<tr>
<td>Number of swollen joints b</td>
<td>12 (5–26)</td>
<td>1 (0–4)</td>
<td>0.0007</td>
</tr>
<tr>
<td>VAS pain by patient c</td>
<td>72 (20–98)</td>
<td>40 (11–80)</td>
<td>0.03</td>
</tr>
<tr>
<td>VAS global by patient a</td>
<td>73 (14–99)</td>
<td>23 (15–45)</td>
<td>0.001</td>
</tr>
<tr>
<td>VAS global by physician</td>
<td>62 (55–85)</td>
<td>17 (13–55)</td>
<td>0.0006</td>
</tr>
<tr>
<td>HAQd</td>
<td>2.6 (1.1–2.8)</td>
<td>0.5 (0.3–1.4)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Results are expressed as median (range).

*Ritchie index: 68 joints were assessed for tenderness.

**Number of swollen joints: 66 joints were assessed for swelling.

*aVAS, Visual Analogue Scale, score can range from 0 to 90 mm.

**dHAQ, Health Assessment Questionnaire, score can range from 0 (no difficulty) to 3 (unable to perform the activity).

Table 3. Monocytes and lymphocyte subsets in RA patients before (d0), after 24 h (d1) and after 6 months of therapy (m6)

<table>
<thead>
<tr>
<th>Lymphocyte Subset</th>
<th>Before therapy d0</th>
<th>After 24 h d1</th>
<th>After 6 months m6</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD14+ (%)</td>
<td>6 (2–7)</td>
<td>5 (2–8)</td>
<td>7 (1–9)</td>
</tr>
<tr>
<td>CD19+ (%)</td>
<td>9 (3–16)</td>
<td>10 (3–16)</td>
<td>10 (4–21)</td>
</tr>
<tr>
<td>CD3+ (%)</td>
<td>80 (63–86)</td>
<td>80 (65–88)</td>
<td>79 (64–88)</td>
</tr>
<tr>
<td>CD4+ (%)</td>
<td>62 (23–77)</td>
<td>67 (24–80)</td>
<td>61 (40–81)</td>
</tr>
<tr>
<td>CD8+ (%)</td>
<td>12 (3–45)</td>
<td>10 (4–31)</td>
<td>9 (2–34)</td>
</tr>
<tr>
<td>CD14+ (×10^6 μl)</td>
<td>503 (178–814)</td>
<td>355 (99–800)*</td>
<td>505 (250–876)</td>
</tr>
<tr>
<td>CD19+ (×10^6 μl)</td>
<td>75 (16–105)</td>
<td>75 (19–111)</td>
<td>82 (20–114)</td>
</tr>
<tr>
<td>CD3+ (×10^6 μl)</td>
<td>1448 (895–3492)</td>
<td>2196 (1007–6428)*</td>
<td>1648 (810–3689)</td>
</tr>
<tr>
<td>CD4+ (×10^6 μl)</td>
<td>877 (428–2688)</td>
<td>1471 (398–4356)*</td>
<td>988 (459–2334)</td>
</tr>
<tr>
<td>CD8+ (×10^6 μl)</td>
<td>373 (92–1365)</td>
<td>523 (158–874)*</td>
<td>387 (105–1256)</td>
</tr>
</tbody>
</table>

CD14+, monocytes; CD19+, B lymphocytes; CD3+, T lymphocytes; CD4+, T-helper (T H) lymphocytes; CD8+, T-cytotoxic (T C)(Ts) suppressor lymphocytes.

Results are expressed as median (range) percentages and as absolute numbers.

*P < 0.05 compared with d0.
Table 4. Percentage and absolute numbers of IL-2-, IL-4- and interferon-γ-positive T lymphocytes in RA patients before (d0), after 24 h (d1) and after 6 months of therapy (m6)

<table>
<thead>
<tr>
<th></th>
<th>Before therapy</th>
<th>After 24 h (d1)</th>
<th>After 6 months (m6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4+ (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-2</td>
<td>13.7 (1.8–42.8)</td>
<td>14 (1.7–36.0)</td>
<td>13 (1.6–36.0)</td>
</tr>
<tr>
<td>Interferon-γ</td>
<td>14 (3.4–31.1)</td>
<td>22.2 (4.9–67.7)*</td>
<td>22.2 (4.9–67.7)*</td>
</tr>
<tr>
<td>IL-4</td>
<td>0.5 (0.1–3.3)</td>
<td>2.8 (0.3–7.2)*</td>
<td>2.8 (0.3–7.2)*</td>
</tr>
<tr>
<td>CD8+ (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-2</td>
<td>9.9 (3.6–31.1)</td>
<td>22.4 (7.5–42.6)*</td>
<td>16.6 (12.4–46.2)*</td>
</tr>
<tr>
<td>Interferon-γ</td>
<td>30.3 (4.8–78.1)</td>
<td>52.9 (10.7–86.2)*</td>
<td>52.9 (10.7–86.2)*</td>
</tr>
<tr>
<td>IL-4</td>
<td>0.8 (0.1–10.1)</td>
<td>2.7 (0.5–6.5)*</td>
<td>2.7 (0.5–6.5)*</td>
</tr>
<tr>
<td>CD4+ (% of CD8+)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-2</td>
<td>114 (98–798)</td>
<td>116 (99–798)</td>
<td>116 (99–798)</td>
</tr>
<tr>
<td>Interferon-γ</td>
<td>133 (5–364)</td>
<td>136 (50–959)*</td>
<td>136 (50–959)*</td>
</tr>
<tr>
<td>IL-4</td>
<td>6 (1–242)</td>
<td>27 (4–215)*</td>
<td>27 (4–215)*</td>
</tr>
<tr>
<td>CD8+ (% of CD4+)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-2</td>
<td>13 (3–131)</td>
<td>51 (1–274)*</td>
<td>51 (1–274)*</td>
</tr>
<tr>
<td>Interferon-γ</td>
<td>38 (6–214)</td>
<td>86 (17–313)*</td>
<td>86 (17–313)*</td>
</tr>
<tr>
<td>IL-4</td>
<td>1 (1–45)</td>
<td>5 (1–214)*</td>
<td>5 (1–214)*</td>
</tr>
</tbody>
</table>

Results are expressed as median (range) percentage cytokine-positive T lymphocytes and as absolute numbers of cytokine-positive T lymphocytes.

*P < 0.05 compared with d0.

Lymphocytic cytokines

Twenty-four hours after treatment with anti-TNFα, the percentages and absolute numbers of IL-4, interferon-γ and IL-2-positive CD4+ T cells were significantly increased (P = 0.05, P = 0.004, P = 0.03, respectively) (Table 4). The rise in IL-4-positive CD4+ T cells was more pronounced than the rise in interferon-γ and IL-2-positive CD4+ T cells, resulting in a significant increase of the T H2/T H1 cytokine ratio in the peripheral blood (for IL-4/IL-2 P = 0.0006 and for IL-4/interferon-γ P = 0.0007, respectively) (Fig. 1). After 6 months, there was still an augmented T H2/T H1 cytokine ratio (for IL-4/IL-2 P = 0.01 and for IL-4/interferon-γ P = 0.003, respectively) (Fig. 1).

For CD8+ T cells, the percentages and absolute numbers of interferon-γ, IL-2- and IL-4-positive cells were also higher after 24 h (P = 0.0001, P = 0.01) (Table 4). In contrast, the absolute numbers of both interferon-γ, IL-2- and IL-4-positive CD8+ T cells did not alter after 6 months of therapy, compared with baseline values (Table 4). The T C2/T C1 cytokine ratio, represented by IL-4/IL-2 and IL-4/interferon-γ, did not change for CD8+ T cells.

Monocytic cytokines

Basal levels and spontaneous production (after 8 h of culture) of IL-1β, IL-6 and TNFα were significantly decreased 24 h after the first administration of anti-TNFα and after 6 months of therapy (P < 0.05) (Fig. 2). Stimulated production (after 8 h of culture with LPS) of IL-1β, IL-6 and TNFα showed a similar rapid decline after 24 h and 6 months of treatment (P < 0.05) (Fig. 2).

Basal IL-12 levels were significantly decreased after 24 h and after 6 months, compared with baseline: 1240 (587–1908) MESF (molecules of equivalent soluble fluorescein) [median (range)] after 24 h and 1120 (736–1763) MESF after 6 months, compared with baseline 1509 (1251–2419) MESF (P = 0.0001, P = 0.003, respectively). In contrast, LPS-stimulated levels of IL-10 rose significantly after 24 h and after 6 months: 1402 (1010–4509) MESF after 24 h and 1170 (800–3180) MESF after 6 months, compared with baseline 970 (607–3323) MESF (P = 0.02, P = 0.01, respectively).

Discussion

It has been shown that therapy with chimeric monoclonal TNFα antibodies leads to a rapid improvement of clinical and biological signs of inflammation in RA [11–14]. There is considerable evidence for two major mechanisms of action: first, there is a reduced cell influx of inflammatory cells into the joints, due to down-regulation of the synovial endothelial adhesiveness and the chemotactic gradient [24–27]; second, there is an inhibition of the pro-inflammatory cytokine cascade. It seems that inflammation in RA, characterized by initial TNF production, which subsequently enhances the production of other pro-inflammatory cytokines such as IL-1, IL-6, IL-8, IL-12 and GM-CSF, can be inhibited by blocking TNFα [26–31].

To our knowledge, this is the first study that investigated the influence of both the short-term (24 h) and long-term (6 months) effect of anti-TNFα therapy on intracellular cytokine profiles in T lymphocytes and monocytes. In contrast, previous studies only evaluated the cytokine profile after a single infusion of anti-TNFα [29–33]. In the present study, we observed a clear reduction of capacity to produce IL-1β, IL-6,
FIG. 2. Intracellular IL-1β (a, b, c), IL-6 (d, e, f) and TNFα (g, h, i) levels in monocytes at time zero (basal), after 8 h of culture (spontaneous production) and after 8 h incubation with LPS (LPS-stimulated production) before therapy (d0), 24 h after the first infusion with anti-TNFα (d1) and after 6 months of therapy (m6). Results are expressed as MESF units.
TNFα and IL-12 in monocytes, immediately after the first infusion of anti-TNFα therapy, which persisted after 6 months of therapy. A flow cytometric intracellular technique was applied in this study to evaluate the altered capacity of monocytes to produce pro-inflammatory cytokines. By blocking produced cytokines intracellularly in the Golgi apparatus, we could avoid complexation of cytokines with their inhibitors, which could be an explanation for some discrepancies in other studies, detecting cytokines in serum with ELISAs: Oshima et al. [31] detected no substantial serum IL-1β, Lorenz et al. [30] observed a decrease in IL-1β production, whereas Charles et al. [32] found no difference in serum IL-1β levels after a single infusion with anti-TNFα antibodies.

Our findings regarding IL-6 production on a short- and longer-term basis are completely in line with previous data that reported decreased serum IL-6 production [29–32]. The decrease in IL-6 could explain the rapid and sustained decrease in acute-phase CRP, also observed in other trials with anti-TNFα therapy [11, 16–18, 32].

On the basis of in vitro experiments, which showed that IL-12 production in RA was partly inhibited by anti-TNFα [34], it was expected that TNFα neutralization would lead to down-regulation of IL-12 production, which was observed in our study.

By serum studies using ELISA assays, not distinguishing immunoreactive from bioactive cytokines, it has not been possible to test equivocally the prediction that TNF blockade will inhibit TNFα production in vivo [31, 32]. In our study, basal, spontaneous and LPS-stimulated TNFα production significantly decreased after 24 h and after 6 months of therapy. In accordance with our findings in peripheral blood monocytes, analysis of synovial tissue before and after treatment showed that TNF blockade was able to reduce TNFα synthesis in the joints [28].

Taken together, this study provides evidence that anti-TNFα blockade can deactivate the pro-inflammatory cytokine cascade, as demonstrated by reductions in serum CRP, IL-1, IL-6, TNFα and IL-12 production.

In addition to the decreased capacity of monocytes to produce pro-inflammatory cytokines, a decrease in absolute monocyte counts was observed, which has been described previously [25, 29–31]. One of the underlying mechanisms could be complement lysis of membrane TNFα-expressing monocytes that bind the anti-TNFα antibody [35]; an alternative explanation would be antibody-dependent cellular cytotoxicity of membrane TNFα-expressing monocytes [35]. Recently, a third mechanism for the decrease in monocyte counts is reported: the capacity of anti-TNFα antibodies to induce apoptosis of monocytes in patients with Crohn’s disease [36].

In addition, we demonstrated an increased capacity of monocytes to produce IL-10, which is in accordance with previous findings of increased serum levels of IL-10 after one infusion of anti-TNFα therapy [31]. This up-regulated IL-10 production can contribute to the correction of the disequilibrium between pro- and anti-inflammatory cytokines in monocytes of RA patients. In vitro experiments reported enhanced pro-inflammatory cytokine (IL-1, IL-6, IL-8, TNFα, GM-CSF) production by blocking IL-10 or a reduction by addition of exogenous IL-10 [37], demonstrating an important role for IL-10 as an anti-inflammatory and immunoregulatory cytokine in monocytes.

In our study, an increased absolute number of lymphocytes was observed: several reports describe an increase of peripheral blood TH1 cells in patients treated with anti-TNFα, associated with the down-regulation of adhesion molecules in the synovium [25, 26, 31, 33], which could be an important factor for inhibiting TH1 cells from migrating into the synovium and inhibiting the release of their TH1 cytokines in the synovium.

We investigated whether TNFα therapy alters the amount of type 1 (represented by IL-2 and interferon-γ) and type 2 (represented by IL-4) CD4+ and CD8+ T lymphocytes within the peripheral blood of RA patients. We could confirm the data presented by Maurice et al. [33] and Lorenz et al. [29], who showed a rise for IL-4-, IL-2- and interferon-γ-positive CD4+ T cells shortly after a single infusion. Moreover, we demonstrated that the increased number of IL-4-, interferon-γ- and IL-2-positive CD4+ T cells was still present after 6 months of anti-TNFα therapy. This increase of TH1 and TH2 cytokine production, at the same time as the up-regulation of IL-10 production and down-regulation of IL-12, is a very interesting and unexpected finding since IL-10 was originally discovered as a TH1-inhibiting cytokine [38–40], whereas IL-12 is known to play an important role in the development of a TH1 response and subsequent interferon-γ production in RA [41].

According to Cope et al. [42], increased IL-2, IL-10 and interferon-γ production in T lymphocytes and improved proliferative responses were reported to occur after prolonged in vitro and in vivo TNFα blockade. Further investigation of patients will allow us to define how TNFα therapy enhances T-cell responsiveness and type 1/type 2 cytokine production. The ratio of TH1/TH2 was increased in comparison with baseline in our study, suggesting a normalization of the initial TH1 cytokine predominance in RA patients towards a more pronounced anti-inflammatory TH2 cytokine balance, in contrast to the study of Maurice. This discrepancy may be partially due to methodological differences (optimal incubation and use of brefeldin A or monensin to block cytokine secretion) [22, 43–46].

In conclusion, repeated administration of anti-TNFα therapy might down-regulate the monocytic capacity to produce pro-inflammatory cytokines and induce a shift to a more pronounced anti-inflammatory TH2 cytokine balance. Further investigation on cytokine profiles in dose–response studies with anti-TNFα therapy has to be performed in order to elucidate the complex mechanisms of anti-TNFα therapy, responsible for the clinical efficacy in rheumatoid arthritis.
We would like to thank Centocor/Schering-plough for providing infliximab. We thank also the staff of the polyclinic of Immunology–Allergology–Rheumatology of the University Hospital of Antwerp for their support. We are especially grateful to P. Claus for her expert administrative and technical assistance.

References

29. Lorenz HM, Antoni C, Valerius T et al. In vivo blockade of TNF\( \alpha \) by intravenous infusion of a chimeric monoclonal TNF\( \alpha \)- antibody in patients with rheumatoid arthritis. Short...


