Evidence That Anti–Tumor Necrosis Factor Therapy With Both Etanercept and Infliximab Induces Apoptosis in Macrophages, But Not Lymphocytes, in Rheumatoid Arthritis Joints

Extended Report

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Objective. Treatment of rheumatoid arthritis (RA) with tumor necrosis factor (TNF) antagonists is highly effective, but their mechanisms of action are not completely clear. Since anti-TNF therapy induces a decrease in synovial cellularity, this study focused on the modulation of RA synovial apoptosis following treatment with either soluble TNF receptor (etanercept) or TNF chimeric monoclonal antibody (infliximab).

Methods. Apoptosis (TUNEL and active caspase 3 staining) and cell surface markers were evaluated by immunohistochemistry in synovial biopsy samples obtained before and after 8 weeks of treatment with etanercept (12 patients) or infliximab (9 patients). We also determined by flow cytometry the in vitro effect of etanercept and infliximab on apoptosis of RA mononuclear cells derived from the synovial fluid (SF) and peripheral blood (PB).

Results. Eight weeks of treatment with etanercept and with infliximab significantly increased synovial apoptosis. This change was accompanied by a significant decrease in the synovial monocyte/macrophage population. The decrease in lymphocyte numbers did not reach statistical significance. In vitro, 24 hours of incubation with either etanercept or infliximab induced apoptosis of the SF monocyte/macrophage population. PB monocyte/macrophages were less susceptible to anti-TNF–mediated apoptosis. No changes in the rate of apoptosis were observed in the lymphocyte population derived from either SF or PB.

Conclusion. In RA patients, both etanercept and infliximab are able to induce cell type–specific apoptosis in the monocyte/macrophage population. This suggests a potential pathway that would account for the diminished synovial inflammation and the decreased numbers of synovial macrophages evident after TNF blockade.

Rheumatoid arthritis (RA) is a chronic inflammatory disease characterized by synovial proliferation and excessive mononuclear infiltration, which lead to the destruction of cartilage and bone. Synovial macrophages, a key cell population in RA, secrete tumor necrosis factor α (TNFα), which induces the production of proinflammatory cytokines and matrix metalloproteinases (MMPs) (1,2). Treatment with TNF antagonists, either infliximab or etanercept, has been shown to be highly clinically efficacious and to delay joint destruc-
tation in RA (3,4). However, the exact mechanism of the antiinflammatory effect is not completely understood. Several mechanisms have been proposed, including decreased cell recruitment at the site of inflammation (5), down-regulation of synovial cytokine expression (6), and reduction of serum levels of MMP-1 and MMP-3 (7,8).

An additional possible mechanism that contributes to the efficacy of TNF-blocking therapy in RA is the regulation of apoptosis, since impaired apoptosis has been implicated as a potential pathogenic mechanism in RA (9) and proapoptotic therapies have been proven to be efficient in different disease models (10). The paucity of synovial apoptotic cells has been associated with synovial NF-κB activation (11), which acts as a potent inhibitor of TNF- and Fas-mediated apoptosis in different cell types, including macrophages and fibroblasts. Both etanercept and infliximab are able to block TNF-induced NF-κB activation (12). Moreover, infliximab has been demonstrated to induce apoptosis of peripheral blood (PB)–derived monocytes (13) and lamina propria–derived T lymphocytes (14) in Crohn’s disease. It has been suggested that this effect is mediated through transmembrane TNF and reverse signaling. To date, both anti-TNF antibodies (15) and soluble TNF receptors (16) are able to bind transmembrane TNF and to consecutively induce intracellular signaling.

In the present study, we demonstrate that 8 weeks of therapy with either etanercept or infliximab induces monocyte/macrophage apoptosis in RA synovium and decreases synovial cellularity. In vitro, both etanercept and infliximab increased the number of apoptotic macrophages, but not lymphocytes, in mononuclear cells isolated from RA synovial fluid (SF) and PB. These data suggest that cell type–specific apoptosis represents one possible pathway that would account for the effects on synovial cellularity and inflammation that are observed after anti-TNF therapy in RA.

PATIENTS AND METHODS

Patients. Twenty-one patients meeting the American College of Rheumatology (ACR; formerly, the American Rheumatism Association) criteria for RA (17) were recruited for this study and were divided into 2 groups. In the first group, 12 patients (10 women and 2 men; median age 47 years [range 35–75 years]) received subcutaneous injections of 25 mg of etanercept twice a week. Eight of these patients were also taking prednisolone, 6 were taking methotrexate, 1 was taking methotrexate and cyclosporine, and 1 was taking sulfasalazine. In the second group, 9 patients (7 women and 2 men; median age 57 years [range 25–69 years]) received infusions of 3 mg/kg of infliximab at week 0, week 2, and week 6. Five of these patients were taking prednisolone, and all 9 patients were taking methotrexate (maximum 20 mg/week). In both groups, stable doses of steroids and disease-modifying antirheumatic drugs were maintained for at least 4 weeks prior to the initiation of anti-TNF therapy and throughout the entire study period. Table 1 shows the clinical characteristics of the patients at study entry.

Overall disease activity was assessed with the Disease Activity Score in 28 joints (DAS28) (18) before and after 12 weeks of treatment. ACR response criteria (19) and European League Against Rheumatism (EULAR) response criteria (20) were used to determine the therapeutic response after 12 weeks of treatment.

Synovial biopsy samples were obtained by arthroscopy (except for 1 patient in group 2 whose second biopsy was obtained during open surgery) from all patients before and after 8 weeks of treatment. The biopsy specimens were taken from an area adjacent to the cartilage–pannus junction, snap-frozen in liquid nitrogen, and maintained at −70°C until sectioned.

SF samples were obtained from 8 additional RA patients and PB samples from 7 additional RA patients. Mononuclear cells from SF (SFMCs) and PB (PBMCs) were isolated by gradient centrifugation using Ficoll-Paque (Pharmacia, Uppsala, Sweden) and stored in liquid nitrogen until assayed.

The ethics committee at the Karolinska Hospital approved all experiments on human cells and tissues. Informed consent was obtained from all study subjects.

Tissue preparation and immunohistochemical analysis. Serial cryostat sections (7 μm) were fixed for 20 minutes with 2% (volume/volume) formaldehyde (Sigma, St. Louis, MO) or for 10 minutes with 100% acetone. Sections were stored at −70°C until analyzed.

Synovial apoptosis was evaluated with the TUNEL technique (21); staining for the active form of caspase 3 was evaluated with an affinity-purified rabbit anti-human active caspase 3 (R&D Systems, Oxford, UK) according to the manufacturer’s instructions. Briefly, blocking of endogenous peroxidase and avidin–biotin was performed, followed by incubation with the primary rabbit polyclonal antibody at a

| Age, median (range) years | 47 (35–75) | 57 (25–69) |
| Disease duration, median (range) months | 138 (60–336) | 72 (7–216) |
| CRP, mean ± SEM mg/liter | 32 ± 4 | 34 ± 10 |
| ESR, mean ± SEM mm/hour | 34 ± 3 | 26 ± 5 |
| DAS28, mean ± SEM | 5.7 ± 0.3 | 6.1 ± 0.3 |
| No. taking other DMARDs | 8 | 9 |
| No. taking corticosteroids | 8 | 5 |

* CRP = C-reactive protein; ESR = erythrocyte sedimentation rate; DAS28 = Disease Activity Score in 28 joints; DMARDs = disease-modifying antirheumatic drugs.
concentration of 0.3 μg/ml. After 24 hours, secondary anti-
rabbit antibody was applied, followed by incubation with
high-sensitivity streptavidin–horseradish peroxidase (HRP)
conjugate and development with 3-amino-9-ethylcarbazole
(AEC) (all reagents from a rabbit HRP–AEC cell and tissue
staining kit obtained from R&D Systems). We also performed
double staining for TUNEL and active caspase 3 using an in
situ cell death detection kit (R&D Systems) according to the
manufacturer’s instructions. Tonsil sections were included as
controls in all experiments.

We characterized the synovial cell phenotype using the
following primary antibodies: anti-CD68 mouse IgG1 (PG-M1;
Dako, Glostrup, Denmark), anti-CD163 mouse IgG1 (Ber-
MAC5; Dako), and anti-CD3 mouse IgG1 (SK7; Becton
Dickinson, Mountain View, CA). Matched IgG isotype con-
trols were included for each marker.

**Microscopic analysis.** All synovial biopsy samples that
had been stained for TUNEL, active caspase 3, and cell surface
markers (CD68, CD163, and CD3) were evaluated semiqual-
titatively in random order by 2 independent observers (AIC
and AKU for apoptosis, AIC and ME for caspase 3, and AIC
and YH for CD markers) who were unaware of the patient’s
identity, biopsy sequence, or therapeutic response. The analy-
sis included all areas of the biopsy tissue, and the final score
represents the mean of the 2 independent observations.

Synovial apoptosis as determined by single TUNEL or
active caspase 3 staining was assessed both semiquantitatively
(4-point scale, where 0 = no positive cells, 1 = occasional
positive cells, 2 = scattered and/or focal positive cells, and 3 =
extensive positive cells) and quantitatively by computer-
assisted image analysis. Double staining was assessed qualita-
tively to confirm the specificity of TUNEL and caspase 3
staining and to describe the apoptotic pattern in the rheuma-
toid synovium. Cell surface markers, including CD3, CD68,
and CD163, were scored using a semiquantitative 4-point scale,
where 0 = no infiltration, 1 = minimal infiltration, 2 =
moderate infiltration, and 3 = marked infiltration, as previ-
ously described (22).

**Cell preparation and flow cytometric analysis.** SFMCs
and PBMCs were cultured in triplicate in RPMI 1640 medium
supplemented with 2 mM l-glutamine, 100 IU/ml of penicillin
and streptomycin, and 20% heat-inactivated fetal calf serum
(all from Gibco, Paisley, UK) and incubated at 37°C in a
humidified atmosphere containing 5% CO₂. Where indicated,
etanercept, infliximab, or control human IgG1κ was added at
the final concentrations of 1, 10, or 100 μg/ml and incubated
for 24 hours.

To test whether etanercept and infliximab were able to
induce apoptosis, cells were double stained with annexin V
and propidium iodide as specified by the manufacturer (R&D
Systems) and analyzed by flow cytometry. Scatter properties
were used to identify the 2 populations of interest, monocyte/
macrophages and lymphocytes. Apoptotic cells were defined as
annexin V single-positive cells, necrotic cells as propidium
iodide single-positive cells, and dead cells as double-positive
cells. Results were expressed as percentages of the total
number of gated monocyte/macrophages and lymphocytes,
respectively.

In order to characterize the type of cells undergoing
apoptosis, cells cultured in the same conditions were stained
with mouse IgG2b peridin chlorophyll protein–conjugated
anti-CD14 (BD Biosciences, San Jose, CA) and with mouse
IgG1 phycoerythrin-conjugated anti-CD3 (BD Biosciences),
icubated with annexin V as specified by the manufacturer
(R&D Systems), and analyzed by flow cytometry. The monoocyte/
macrophages and lymphocytes were identified by fluorochrome-labeled anti-CD14 and anti-CD3 antibodies, respec-
tively, and by the scatter properties determined by flow
cytometry. The gated populations were then analyzed for the
surface expression of annexin V. Apoptosis was quantified as
the mean percentage of annexin V–positive CD14+ cells from
the total number of CD14+ cells and the mean percentage of
annexin V–positive CD3+ cells from the total number of
CD3+ cells.

**Statistical analysis.** Statistical analysis was performed
using analysis of variance (ANOVA), with the Kruskal-Wallis
ANOVA and the Friedman ANOVA when appropriate, fol-
lowed by Wilcoxon’s or Dunnett’s post hoc analysis. Correla-
tions were assessed using Spearman’s rank correlation test. P
values less than 0.05 were considered statistically significant.

**RESULTS**

**Clinical response.** Both the clinical and labora-
tory parameters of disease activity improved after 12
weeks of treatment, with a significant reduction in all
parameters assessed ($P < 0.05$), including the DAS28
score, C-reactive protein (CRP) level, and erythrocyte
sedimentation rate (ESR), in the etanercept-treated
group. After 12 weeks of infliximab treatment, the
DAS28 score decreased significantly, whereas an appar-
ent decrease in the CRP and ESR values did not reach
statistical significance. Using the ACR 20% improve-
ment criteria (ACR20), 9 of the 12 patients in the
etanercept group improved and 6 of the 9 patients in the
infliximab group improved. Using the EULAR response
criteria, 2 patients in the etanercept group were nonre-
ponders, 6 were moderate responders, and 4 were good
responders, whereas in the infliximab group, 2 patients
were nonresponders, 5 were moderate responders, and 2
were good responders.

**Findings of immunohistochemical analyses.** After
8 weeks of anti-TNF treatment with either etaner-
cept or infliximab, the level of synovial apoptosis signif-
icantly increased ($P < 0.05$), as evaluated by single
staining with TUNEL (Figure 1A) and with active
caspase 3 (Figure 1B). TUNEL-positive cells were
present in both the lining and the sublining layers,
mainly in mononuclear cells. Caspase 3 activity was
detected in mononuclear cells as well as in some isolated
fibroblast-like cells and endothelial cells.

Double staining for both etanercept and inflix-
imab identified mainly double-positive cells and caspase
3 single-positive cells; TUNEL single-positive cells were
Figure 1. Induction of apoptosis in rheumatoid synovium by etanercept and infliximab. A, Treatment with a–c, etanercept and d–f, infliximab induces apoptosis. Serial sections of frozen synovial biopsy tissues from 1 patient before (a) and after (b) etanercept treatment and from 1 patient before (d) and after (e) infliximab treatment show diaminobenzidine (brown) immunoperoxidase staining for TUNEL (hematoxylin counterstained). Insets show intermediate fluorescence images of the same biopsy samples. (Original magnification × 200; bar = 15 μm). Graphs show results from image analysis of the biopsy tissues before and after etanercept (c) and infliximab (f) treatment. Values are the mean ± SEM. * = P < 0.05. B, Synovial apoptosis induced by both a–c, etanercept and d–f, infliximab is mediated by caspase 3. Serial sections of frozen synovial biopsy tissues from 1 patient before (a) and after (b) etanercept treatment and from 1 patient before (d) and after (e) infliximab treatment show 3-amino-9-ethylcarbazole (red) immunostaining for active caspase 3 (hematoxylin counterstained) (original magnification × 200; bar = 15 μm). Graphs show results from image analysis of the biopsy tissues before and after etanercept (e) and infliximab (f) treatment. Values are the mean ± SEM. * = P < 0.05.
also detected, but to a lesser extent (Figure 2A). The fold increase in synovial apoptosis as evaluated by TUNEL may be associated with the clinical response to therapy, since the ACR20 responders in both groups appeared to have a greater increase in apoptosis levels as compared with the ACR20 nonresponders, even though the differences did not reach statistical significance (mean ± SEM fold increase 5.2 ± 1.9 versus 1.9 ± 0.4 in the etanercept group, and 2.6 ± 1.2 versus 4.6 ± 2.6 in the infliximab group) (Figure 2B). The same tendency was observed for active caspase 3 staining in the etanercept-treated group, with a mean ± SEM fold increase of 4.2 ± 1.5 in the responders.
Figure 3. Decrease in synovial cellularity induced by etanercept and infliximab. A, Treatment with etanercept decreases synovial cellularity. Frozen sections of synovial biopsy tissues from 1 patient treated with etanercept show diaminobenzidine (brown) immunoperoxidase staining (hematoxylin counterstained) for surface marker CD68 before (a) and after (b) treatment, for CD163 before (d) and after (e) treatment, and for CD3 before (g) and after (h) treatment (original magnification × 200; bar = 15 μm). Graphs show results from semiquantitative evaluation of biopsy tissues for CD68 (c), CD163 (f), and CD3 (i) before and after etanercept treatment. Values are the mean ± SEM. * = P < 0.05. B, Treatment with infliximab decreases synovial cellularity. Frozen sections of synovial biopsy tissues from 1 patient treated with infliximab show diaminobenzidine (brown) immunoperoxidase staining (hematoxylin counterstained) for surface marker CD68 before (a) and after (b) treatment, for CD163 before (d) and after (e) treatment, and for CD3 before (g) and after (h) treatment (original magnification × 200; bar = 15 μm). Graphs show results from semiquantitative evaluation of biopsy tissues for CD68 (c), CD163 (f), and CD3 (i) before and after infliximab treatment. Values are the mean ± SEM. * = P < 0.05.
and 2.5 ± 0.3 in the nonresponders. However, in the infliximab-treated group, no such differences in active caspase 3 staining were observed, with a mean ± SEM fold increase of 1.5 ± 0.2 in the responders and 1.7 ± 0.2 in the nonresponders (Figure 2C).

The number of CD68+ cells decreased significantly following both treatments, while the number of CD163+ macrophages decreased significantly only in the etanercept group. The number of CD3+ cells did not change significantly after either treatment (Figure 3).

**Findings of flow cytometric analyses.** To further evaluate the eventual proapoptotic effect of the 2 TNF-blocking agents, we first evaluated RA SFMCs treated in vitro with etanercept and infliximab for 24 hours by double labeling the cells with annexin V and propidium iodide. Treatment with both etanercept and infliximab induced an increase in the number of apoptotic macrophages (annexin V single-positive cells) without changing the number of necrotic (propidium iodide single-positive cells) or dead (annexin V/propidium iodide double-positive cells) macrophages, as compared with the control cultures (Figures 4A and 5A). No significant differences in the number of annexin V–positive and/or propidium iodide–positive cells were observed in the lymphocyte population (Figures 4B and 5B).

To characterize the apoptotic cell type, we evaluated annexin V expression following cell typing using the cell surface markers CD14, for monocyte/macro-
phages, and CD3, for T lymphocytes. Quantitative analysis of CD14+ SFMCs treated with etanercept (n = 4) revealed a mean ± SEM increase in annexin V expression of 2.9 ± 0.3 at 1 μg/ml and 2.1 ± 0.4 at 10 μg/ml as compared with control IgG1-k-treated cells (P < 0.05); the increase of 1.4 ± 0.2 observed following treatment with 100 μg/ml of etanercept did not reach statistical significance (Figures 4C and D).

Treatment of SFMCs with infliximab (n = 4) induced a similar increase in annexin V expression in the CD14+ SFMCs to a mean of 1.4 ± 0.1 at 1 μg/ml and 1.4 ± 0.2 at 10 μg/ml as compared with control IgG1-k-treated cells (P < 0.05); the increase observed following treatment with 100 μg/ml of infliximab did not reach statistical significance (Figures 5C and D).

Consistent with the immunohistochemistry results, we did not observe any significant differences in the number of CD3+ cells expressing annexin V following either etanercept (Figure 4D) or infliximab (Figure 5D) treatment.

To test whether the anti-TNF proapoptotic effect is restricted to the synovium, we evaluated the effect of both etanercept and infliximab on PBMCs obtained from 7 RA patients. Both etanercept and infliximab appeared to increase annexin V expression in CD14+ PBMCs (Figure 6) to a maximum mean ± SEM of 1.7 ± 0.4 and 1.5 ± 0.2, respectively, as compared with control cultures, but the differences did not reach statistical significance. Annexin V expression in the CD3+ PBMC population remained essentially unchanged following
treatment with either etanercept or infliximab (Figure 6).

DISCUSSION

Anti-TNF therapy in RA patients is highly clinically efficacious, but the exact mechanisms of action are still incompletely understood. We have demonstrated herein that there was a significant increase in synovial apoptosis following treatment with both etanercept and infliximab in patients with RA.

Our in vivo investigation was designed as an open-label study that, theoretically, has some drawbacks, such as a placebo effect and expectation bias. However, previous studies revealed no changes in serial synovial biopsy samples obtained from patients receiving placebo or ineffective clinical therapy (23–25). We report here beneficial clinical responses similar to those previously demonstrated in double-blind placebo-controlled trials (26,27), accompanied by significant changes in synovial phenotype. These data, taken together, support the view that the observed changes in serial synovial biopsy samples are the consequence of a therapeutic biologic effect and not a placebo effect. We also took great care to blind the readers of the biopsy samples as to both the therapy and biopsy sequence. This precaution should also have reduced the risks of expectation bias.

The immunohistochemistry results demonstrated that etanercept treatment decreases the number of monocyte/macrophages, which were evaluated as both CD68+ and CD163+ cells, without changing the number of CD3+ cells. This suggests a selective effect of etanercept on macrophages. In the infliximab-treated group, a similar significant decrease in the CD68+ cell number was observed, while the CD3+ cell number tended to decrease but did not reach statistical significance. These results are consistent with those of a recent placebo-controlled study using 3 mg/kg of infliximab (28).

The reduction in the number of monocyte/macrophages after etanercept and infliximab treatment was accompanied by an up-regulation of synovial apoptosis, as detected by both TUNEL and active caspase 3 staining. The TUNEL technique, a classic method for detecting apoptosis in sample tissues, has a high sensitivity but a low specificity, and therefore a second detection method is required for definite identification of the apoptotic process (29). Thus, we chose to investigate the presence of the active form of caspase 3 both in a single-staining procedure (to be able to quantify the results by image analysis) and a double-staining procedure (to be able to confirm the TUNEL specificity). Consistent with the findings of a previous study (30),

Figure 6. Induction of apoptosis in peripheral blood mononuclear cells (PBMCs) by A, etanercept and B, infliximab. Both etanercept and infliximab were less potent in promoting apoptosis of PBMCs. Both treatments caused an increase in the annexin V expression of CD14+ PBMCs (P not significant versus control) and had essentially no effect on CD3+ cells. Values are the mean ± SEM and are expressed as the ratio of annexin V–positive cells in the experimental cultures to those in the control cultures (fold).
synovial apoptosis correlated with the macrophage number but not with individual macrophage-derived cytokines, such as TNF, interleukin-1α (IL-1α), and IL-1β (data not shown), which suggests a concerted action of these molecules in defining cell susceptibility to apoptosis. The implication of apoptotic cell death as a potential mechanism of action of anti-TNF drugs is emphasized by the tendency toward greater increases in the apoptotic index in the ACR20 responders versus the ACR20 nonresponders, although larger numbers of observations will be needed in order to substantiate the eventual usefulness of apoptotic markers as a way of discriminating between responders and nonresponders to TNF blockade.

Consistent with our in vivo findings, we demonstrated that treatment with both etanercept and infliximab induces apoptosis in CD14+ SFMCs and, to a lesser extent, in PBMCs from patients with RA. Our findings are consistent with previous reports of infliximab-induced apoptosis of monocytes derived from patients with Crohn's disease (13). To date, there have been no similar investigations into the effect of etanercept on monocytes. Interestingly, we did not observe an effect of either of the tested drugs on the CD3+ lymphocyte population, which suggests a specific pro-apoptotic effect on RA-derived macrophages. The lack of effect of etanercept on lymphocyte survival has also been documented in Crohn's disease (12).

Previous reports suggested that infliximab is able to induce apoptosis in activated, but not resting, lymphocytes (12,14). A potential explanation for the observed lack of effect on T cells is the phenotype of joint-derived lymphocytes from patients with RA, which are known to differ substantially from gut-derived lymphocytes from patients with Crohn's disease. Gut-derived lymphocytes from Crohn's disease patients are highly activated and are able to produce high levels of cytokines (31). We and other investigators (32–34) have demonstrated that lymphocytes derived from both synovial tissue and SF cells express low levels of cytokines and show signs of anergy. Moreover, repeated in vitro treatment with TNF, a setting that mimics the long-term exposure to TNF in the RA synovial environment, suppresses T cell activity (35). Even though anti-TNF therapy is able to reverse to some extent the peripheral T cell reactivity (36), it might be that the complex arthritic milieu influences synovial T cell reactivity at the site of inflammation to such an extent that it might not fully recover. The lack of effect of high concentrations of the 2 drugs has previously been observed for etanercept with regard to cytokine suppression (37,38) and for infliximab with regard to apoptosis induction in the presence of minute amounts of TNF (39). These findings suggest the presence of an escape mechanism at high concentrations of the anti-TNF agents, possibly mediated through redundant proinflammatory pathways.

Both infliximab and etanercept treatment increased the proportion of apoptotic cells in CD14+ PBMCs, but the effect was less potent compared with that in SFMCs, suggesting that the particular milieu of the rheumatoid joint influences monocyte susceptibility to apoptosis. Keeping in mind the differences between monocyte populations in SF and PB, we propose several potential mechanisms to explain the proapoptotic effect of the anti-TNF therapy. First, as previously demonstrated, both anti-TNF antibodies (15) and soluble TNF receptors (16) can bind membrane TNF and transmit an intracellular signal, and it has been speculated that apoptosis of the target cell could be a result of this pathway (40). Monocytes in synovial tissue and SF have an activated phenotype, with sustained cytokine secretion, but with only scarce translation detectable in PB (41); these observations correlate with the lower potency of the drugs to induce apoptosis in PBMCs. Second, it has been shown that TNF protects monocytes from death receptor-mediated apoptosis through up-regulation of FLIP (42). FLIP is abundantly expressed in RA monocytes derived from synovial tissue and SF and, to a lesser extent, in peripheral blood monocytes (43). Our preliminary data suggest that there is a decrease in synovial FLIP expression following treatment with both etanercept and infliximab, indicating a possible FLIP-mediated apoptotic mechanism (Catrina AI: unpublished observations). Third, activation of NF-κB, a common feature of the RA synovium, protects monocyte-derived macrophages against apoptosis. Both etanercept and infliximab are able to inhibit NF-κB, and, as previously demonstrated, inhibition of NF-κB induces macrophage apoptosis (44).

Our findings differ in part from those of a previous study showing decreased synovial cellularity after treatment with infliximab for 48 hours and for 28 days, without changes in the number of apoptotic cells (28). At least for the early time point in that study, it is possible that the timing of the arthroscopy may account for the differences. Even though apoptosis induction is a rapid event, altering the status of an antiapoptotic milieu such as the RA synovium requires complex changes. The effects of such complex events may become evident as a change in apoptotic cells only at later time points, including the 8 weeks of treatment (with 2 weeks after the last infliximab injection) used in the present study.
This approach is supported by a recent study in Crohn’s disease demonstrating an increased level of apoptosis after 10 weeks of infliximab therapy as compared with baseline (45). It also appears that in the study conducted by Smeets et al (28), following 48 hours of infliximab treatment, the number of apoptotic cells corrected for the total number of synovial cells may have increased, although not statistically significantly, since they describe a global decrease in cellularity (except for CD22) with same number of TUNEL-positive cells per tissue area. However, we could exclude neither a possible Type I error in our study (due to multiple comparisons, although we corrected for multiple comparisons) nor a Type II error in the previous study (due to a low number of patients). Further investigations including a larger number of patients, different treatment durations, and different biopsy times are needed in order to address these issues.

In conclusion, our data suggest that therapy with both etanercept and infliximab increases synovial monocyte/macrophage apoptosis in RA patients, thereby providing an alternative and complementary pathway to account for the decreased macrophage activity and inflammation observed after anti-TNF therapy.

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