Effects of Infliximab Treatment on Rheumatoid Synovial Tissue

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ABSTRACT. Several studies have shown decreased synovial inflammation after treatment of patients with rheumatoid arthritis (RA) with infliximab. Recent data indicate that these effects can be detected as soon as 48 hours after first infusion. Although there are strong indications that infliximab exerts its effects in patients with Crohn's disease by induction of apoptosis in the gut, there are as yet no studies that convincingly show the same mechanism of action in RA. Conceivably, neutralization of tumor necrosis factor-α may be sufficient to induce clinical improvement in RA, even without induction of apoptosis at the site of inflammation. This hypothesis could explain the observation that both infliximab and etanercept are effective in RA, whereas apoptosis induction by infliximab might be required in Crohn's disease. Future studies should focus on the evaluation of apoptosis within the first 48 hours after initiation of therapy in RA to exclude the possibility that the effects occur very early after infliximab infusion. (J Rheumatol 2005;32 Suppl 74:31-34)

Key Indexing Terms:
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CROHN'S DISEASE

Rheumatoid arthritis (RA) is a chronic disease characterized by synovial inflammation, leading to destruction of cartilage and bone[1,2]. As RA is an inflammatory disease that primarily involves the synovium, it can be expected that examination of synovial tissue samples may provide insight into the pathogenesis of the disease and the mechanism of action of targeted therapies. Hence, descriptive studies of rheumatoid synovium may contribute to an understanding of the events that take place *in vivo* and complement and validate results from *in vitro* studies and animal models. This review describes the changes in synovial tissue samples of RA patients treated with infliximab.

RHEUMATOID SYNOVIAL TISSUE

The synovium lines the noncartilaginous surfaces of the synovial joints and provides nutrients to avascular structures, such as cartilage. It consists of an intimal lining layer that normally contains one to 3 layers of cells, without an underlying basal membrane overlying the synovial stroma, termed synovial sublining[3]. The intimal lining layer is formed by 2 types of cells: intimal macrophages and fibroblast-like synoviocytes[4]. The synovial sublining is formed by a loose collagen fiber network with fibroblasts, fat cells, and scattered blood vessels.

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In RA, the synovium is hypertrophic and edematous. Rheumatoid synovial tissue is characterized by marked intimal lining hyperplasia and by accumulation of T cells, plasma cells, macrophages, B cells, neutrophils, mast cells, natural killer cells, and dendritic cells in the synovial sublining[3]. Of note, most of the macrophages in actively inflamed joints are found in the synovial sublining[5]. In addition, there is neoangiogenesis, vascular congestion, and fibrin deposition. Recruitment of inflammatory cells, local retention, cell proliferation, and impaired apoptosis may all contribute to the increased cellularity of the rheumatoid synovium.

RELATIONSHIP BETWEEN SYNOVIAL PATHOLOGY AND DISEASE ACTIVITY

An important question is whether the features of inflamed synovial tissue are related to measures of disease activity. A cross-sectional study in patients with RA using stepwise multiple regression analysis revealed that scores for knee pain are particularly associated with the number of macrophages in the synovial sublining, as well as the expression of macrophage-derived cytokines [such as tumor necrosis factor-α (TNF-α) and interleukin 6] in biopsy samples from that same joint[6]. Similarly, macrophage numbers are increased in clinically involved joints compared to clinically uninvolved joints of the same patients[7]. It has also been suggested that there is a positive correlation between cell counts for macrophages in the synovial sublining on the one hand and radiographic signs of joint destruction 6 years after the biopsy procedure on the other.

To provide a greater understanding of the changes in the synovium alongside clinical response, we analyzed serial synovial tissue samples in a randomized trial using a known effective therapy[8]. Patients received either prednisolone according to the COBRA regimen[9] or placebo

for 2 weeks. Synovial biopsies were taken before initiation of treatment and at 2 weeks, and tissue sections were analyzed for a variety of immunohistologic markers. Each of the endpoints was statistically analyzed using an analysis model of covariance. Macrophages were identified as the best biomarker associated with the clinical response to corticosteroids[10]. In keeping with this concept a predominant reduction in macrophage numbers of the synovial membrane has been reported in patients who achieved clinical remission induced by use of various disease modifying antirheumatic drugs[11]. Thus, various effective antirheumatic drugs with discrete mechanism of action appear to have an ultimate effect on macrophages, leading to clinical improvement. TNF-α is predominantly produced by activated macrophages in the inflamed synovium.

EFFECTS OF INFLIXIMAB TREATMENT ON SYNOVIAL CELL INFILTRATE

Studies of the effects of anti-TNF treatment with infliximab in RA have shown that synovial cellularity infiltration is reduced 4 weeks after a single infusion of 10 mg/kg infliximab[12]. In the same way, the number of infiltrating cells was decreased 2 weeks after a single infusion of 10 mg/kg infliximab[13].

A recent study analyzed the effects of infliximab in RA synovial tissue even earlier, after initiation of the usual starting dose for this disease (3 mg/kg)[14]. To this end RA patients were randomized to receive either infliximab or placebo. All patients were subjected to an arthroscopic synovial biopsy procedure of a swollen knee, wrist, or ankle joint directly before initiation of treatment. A second arthroscopic synovial biopsy procedure of the same joint was performed 48 hours after the first. After second arthroscopy the patients who had initially received placebo were also treated with infliximab (3 mg/kg) in an extension study. A second administration of 3 mg/kg infliximab was administered to all patients on Day 15. A third arthroscopy was performed on Day 28. Thus, this was a placebo-controlled trial during 48 hours followed by an open extension study during 28 days. There was a significant reduction in the number of intimal macrophages already 48 hours after initiation of infliximab therapy, whereas there were no significant changes in the placebo group (Figure 1)[14]. Similarly, there was a marked decrease in the number of sublining macrophages as well as trends towards reduced T cell and plasma cell

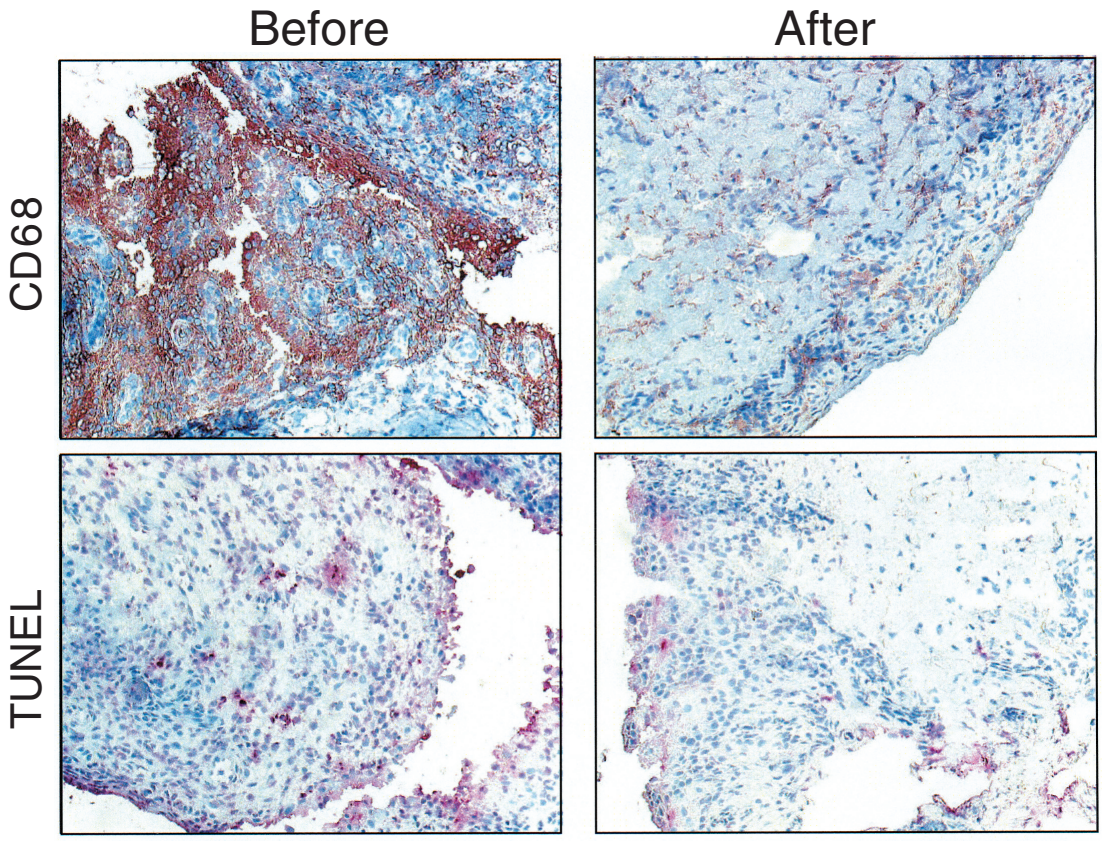

Figure 1. TUNEL-positive apoptotic cells after treatment with infliximab. Paired synovial tissue samples obtained from a representative patient with rheumatoid arthritis before and 48 hours after initiation of infliximab therapy (3 mg/kg), showing a marked decrease in the number of CD68-positive macrophages (red-brown staining), but no increase in TUNEL-positive apoptotic cells (red staining) after treatment.

numbers. The same trends were observed after 28 days. Of interest, decreased cellularity was found in clinical responders, but not in patients who did not exhibit a decrease in DAS 28 > 1.2 after 28 days.

DOES APOPTOSIS INDUCTION EXPLAIN THE RAPID EFFECT OF INFLIXIMAB?

The rapidity of the effects of TNF-α blockade compared to conventional treatment with disease modifying antirheumatic drugs is remarkable. Clinical improvement has been described as early as one week after initiation of treatment with infliximab[15]. As discussed above marked changes in synovial biopsy samples can be detected 48 hours after first infusion. How can this be explained? Several studies have shown that impaired apoptosis plays a role in the development of synovial hyperplasia[16]. Few apoptotic cells are found in the rheumatoid synovium[17,18], despite the presence of fragmented DNA[17,19,20]. One might hypothesize that infliximab treatment reduces the synovial cell infiltrate by induction of apoptosis. This notion is supported by *in vitro* work using murine myeloma cells expressing mutant human TNF-α[21]. Infliximab may kill these cells by both antibody-dependent cellular toxicity and complement-dependent cytotoxicity effector mechanisms. It has also been suggested that *in vitro* treatment of peripheral blood monocytes from patients with Crohn's disease with infliximab leads to induction of apoptosis through activation of caspase 8 and the mitochondrial pathway[22]. Another study has shown that infliximab, but not etanercept, may induce apoptosis in peripheral and lamina propria lymphocytes[23]. Infliximab activated caspase 3 in a time-dependent manner. Further support for apoptosis induction as an explanation for decreased inflammation comes from clinical studies in patients with Crohn's disease. These patients had an increase in cells exhibiting DNA fragmentation in colonic biopsies 24 hours after first infusion of 5 mg/kg infliximab[24]. It has been proposed that induction of apoptosis by anti-TNF-α antibodies, but not by soluble TNF receptors, could explain the difference in therapeutic efficacy between infliximab and etanercept in Crohn's disease[25,26].

To determine whether increased apoptosis contributes to the efficacy of infliximab in RA, we performed TUNEL assays, a sensitive technique to detect apoptotic cells, on synovial tissue samples taken before initiation of treatment and after 48 hours and 28 days[14]. To our surprise, we did not detect any increase in the number of TUNEL-positive cells after treatment with infliximab at a dose of 3 mg/kg (Figure 1). The results were confirmed by caspase-3 staining and electron microscopy. Hence, this study did not provide support for the notion that the reduction in synovial cell infiltration after infliximab therapy could be explained by the induction of apoptosis in the rheumatoid synovium. However, we cannot exclude the possibility that apoptosis induction occurs even earlier and that all apoptotic cells had disappeared after 48 hours. Support for this view comes from a recent study showing an increase in plasma nucleosomes, which are generated during cell death, as early as 2 hours after infliximab infusion[27]. It is unclear, however, which dose was given in that study. Our preliminary work so far did not provide evidence for apoptosis induction in peripheral blood monuclear cells 1 hour or 24 hours after completion of the infliximab infusion at 3 mg/kg (C.A. Wijbrandts, *et al*; unpublished observations). Data on synovial tissue at these time points are as yet not available.

The discrepancy with results in patients with Crohn's disease might also be explained by difference in dosage of infliximab or by differences in disease pathogenesis. It is possible that neutralization of the effects of TNF-α may be sufficient to induce clinical improvement in RA, even without induction of apoptosis at the site of inflammation[14]. This could explain the fact that treatments with anti-TNF-α antibodies and soluble TNF receptors are both effective in RA, unlike the effects in Crohn's disease, where induction of apoptosis may be crucial to obtain clinical efficacy[25]. It is possible that higher infliximab dosages would be able to induce apoptosis in RA patients, but perhaps this is not essential to achieve clinical improvement.

EFFECTS OF INFLIXIMAB TREATMENT ON CELL MIGRATION

Reduced synovial cellularity after infliximab treatment may be explained in part by decreased cell migration[12,13,28]. Treatment with infliximab results in decreased expression of adhesion molecules in synovial tissue[12], reduced levels of circulating soluble adhesion molecules[28], and diminished chemokine levels in the synovium[13]. These factors are intimately involved in migration of inflammatory cells towards the inflamed compartment, as well as in the retention of these cells at the site of inflammation. In addition, an inhibitory effect of infliximab therapy on synovial angiogenesis could further reduce cell migration to the joints[29]. The notion that anti-TNF therapy may reduce cell migration is supported by a study showing a decreased signal on gamma-camera images of the joints after intravenous injection of ^{111}In-labeled granulocytes in patients treated with infliximab[13]. In addition, a rapid and sustained increase in circulating lymphocytes has been observed after infusion of infliximab, in accord with decreased cell traffic to the inflamed joints[28]. An obvious question is whether reduced migration and inhibition of retention of cells alone could be sufficient to induce a clinical effect. Initial experience with a chemokine receptor blockade suggests that this may indeed be the case[30,31].

CONCLUSION

Infliximab treatment induces rapid changes in the rheumatoid synovium. The effects of anti-TNF therapy may be explained in part by reduced cell migration and

inhibition of retention of inflammatory cells in the inflamed synovium. When dosages of 3 mg/kg are used, there are no clear signs of apoptosis induction 48 hours or 28 days after the infusion. There remain several questions. Although there is strong circumstantial evidence, direct proof that anti-TNF treatment does inhibit monocyte migration is not yet available. It is unclear where the synovial cells go after infliximab infusion: do they recirculate or die at other sites? How much trafficking is there between the synovium and the circulation? We also need more investigation on the effects of infliximab within the first 48 hours after initiation of treatment. Finally, it might be difficult to establish whether tiny numbers of apoptotic cells that may be missed by morphological examination are present for prolonged periods of time, ultimately leading to reduced cellularity. Nevertheless, initial studies suggest that there is a difference between tissue response in Crohn's disease compared to RA. This line of investigation may lead to a clue about differences in the pathogenesis of these disorders.

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