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:
INFLIXIMAB
TUMOR NECROSIS FACTOR
RHEUMATOID ARTHRITIS
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

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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

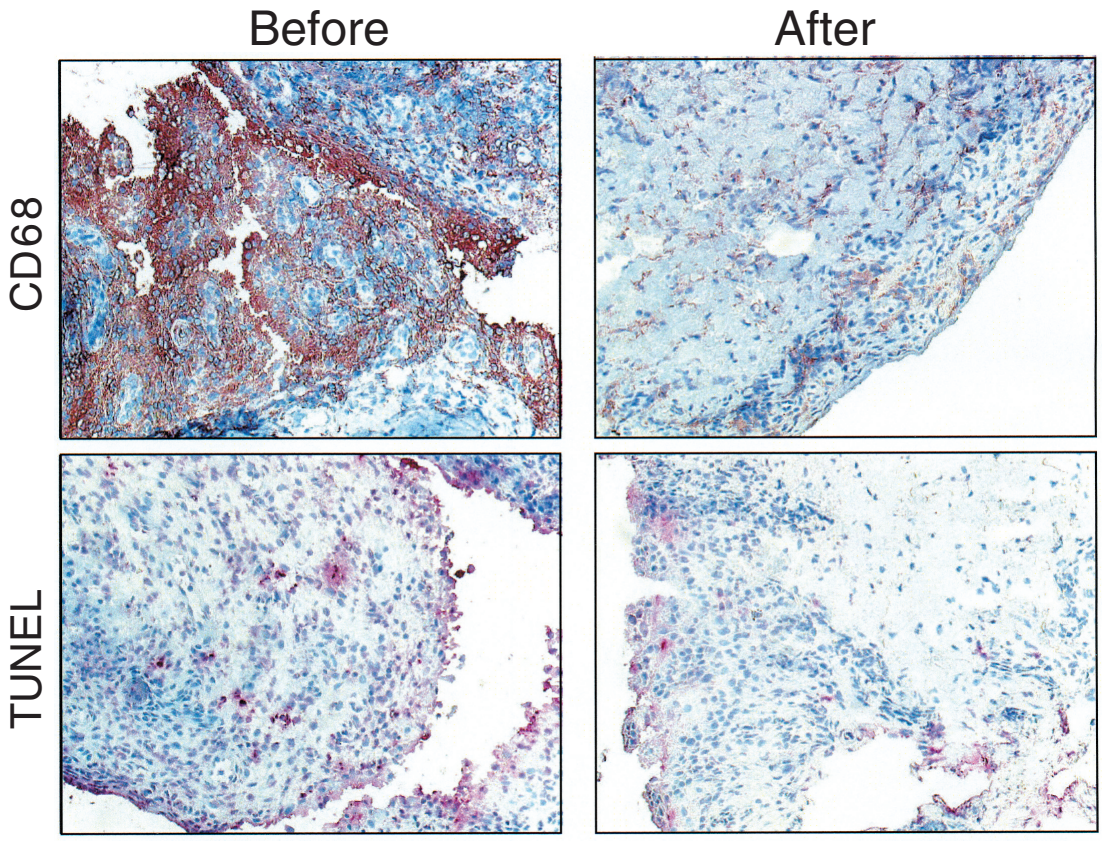

Figure 1. TUNEL-positive apoptotic cells after treatment with infliximab. Paired synovial tissue samples obtained from a representative patient with rheumatoid arthritis before and 48 hours after initiation of infliximab therapy (3 mg/kg), showing a marked decrease in the number of CD68-positive macrophages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

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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

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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.

REFERENCES

1. Tak PP, Bresnihan B. The pathogenesis and prevention of joint damage in rheumatoid arthritis: advances from synovial biopsy and tissue analysis. Arthritis Rheum 2000;43:2619-33.
2. Firestein GS. Evolving concepts of rheumatoid arthritis. Nature 2003;423:356-61.
3. Tak PP. Examination of the synovium and synovial fluid. In: Firestein GS, Panayi GS, Wollheim FA, editors. Rheumatoid arthritis. Frontiers in pathogenesis and treatment. New York: Oxford University Press; 2000:55-68.
4. Edwards JC, Willoughby DA. Demonstration of bone marrow derived cells in synovial lining by means of giant intracellular granules as genetic markers. Ann Rheum Dis 1982;41:177-82.
5. Smeets TJ, Barg EC, Kraan MC, Smith MD, Breedveld FC, Tak PP. Analysis of the cell infiltrate and expression of proinflammatory cytokines and matrix metalloproteinases in arthroscopic synovial biopsies: comparison with synovial samples from patients with end stage, destructive rheumatoid arthritis. Ann Rheum Dis 2003;62:635-8.
6. Tak PP, Smeets TJ, Daha MR, et al. Analysis of the synovial cell infiltrate in early rheumatoid synovial tissue in relation to local disease activity. Arthritis Rheum 1997;40:217-25.
7. Kraan MC, Versendaal H, Jonker M, et al. Asymptomatic synovitis precedes clinically manifest arthritis. Arthritis Rheum 1998;41:1481-8.
8. Gerlag DM, Haringman JJ, Smeets TJ, et al. Effects of oral prednisolone on biomarkers in synovial tissue and clinical improvement in rheumatoid arthritis. Arthritis Rheum 2004;50:3783-91.
9. Boers M, Verhoeven AC, Markusse HM, et al. Randomised comparison of combined step-down prednisolone, methotrexate and sulphasalazine with sulphasalazine alone in early rheumatoid arthritis. Lancet 1997;350:309-18.
10. Gerlag DM, Haringman JJ, Smeets TJ, et al. Effects of oral prednisolone on biomarkers in synovial tissue and clinical improvement in rheumatoid arthritis. Arthritis Rheum 2004;50:3783-91.
11. Smith MD, Kraan MC, Slavotinek J, et al. Treatment-induced remission in rheumatoid arthritis patients is characterized by a reduction in macrophage content of synovial biopsies. Rheumatology Oxford 2001;40:367-74.
12. Tak PP, Taylor PC, Breedveld FC, et al. Decrease in cellularity and expression of adhesion molecules by anti-tumor necrosis factor alpha treatment in patients with rheumatoid arthritis. Arthritis Rheum 1996;39:1077-81.
13. Taylor PC, Peters AM, Paleolog E, et al. Reduction of chemokine levels and leukocyte traffic to joints by tumor necrosis factor alpha blockade in patients with rheumatoid arthritis. Arthritis Rheum 2000;43:38-47.
14. Smeets TJ, Kraan MC, van Loon ME, Tak PP. Tumor necrosis factor alpha blockade reduces the synovial cell infiltrate early after initiation of treatment, but apparently not by induction of apoptosis in synovial tissue. Arthritis Rheum 2003;48:2155-62.
15. Elliott MJ, Maini RN, Feldmann M, et al. Randomised double-blind comparison of chimeric monoclonal antibody to tumour necrosis factor alpha (cA2) versus placebo in rheumatoid arthritis. Lancet 1994;344:1105-10.
16. Tak PP, Firestein GS. Apoptosis in rheumatoid arthritis. In: Winkler JD, editor. Apoptosis and inflammation. Basel: Birkhauser Publishing Ltd.; 1999:149-62.
17. Nakajima T, Aono H, Hasunuma T, et al. Apoptosis and functional Fas antigen in rheumatoid arthritis synoviocytes. Arthritis Rheum 1995;38:485-91.
18. Matsumoto S, Muller-Ladner U, Gay RE, Nishioka K, Gay S. Ultrastructural demonstration of apoptosis, Fas and Bcl-2 expression of rheumatoid synovial fibroblasts. J Rheumatol 1996;23:1345-52.
19. Firestein GS, Yeo M, Zvaifler NJ. Apoptosis in rheumatoid arthritis synovium. J Clin Invest 1995;96:1631-8.
20. Catrina AI, Ulfgren AK, Lindblad S, Grondal L, Klareskog L. Low levels of apoptosis and high FLIP expression in early rheumatoid arthritis synovium. Ann Rheum Dis 2002;61:934-6.
21. Scallon BJ, Moore MA, Trinh H, Knight DM, Ghrayeb J. Chimeric anti-TNF-alpha monoclonal antibody cA2 binds recombinant transmembrane TNF-alpha and activates immune effector functions. Cytokine 1995;7:251-9.
22. Lugering A, Schmidt M, Lugering N, Pauels HG, Domschke W, Kucharzik T. Infliximab induces apoptosis in monocytes from patients with chronic active Crohn's disease by using a caspase-dependent pathway. Gastroenterology 2001;121:1145-57.
23. Van den Brande JM, Braat H, van den Brink GR, et al. Infliximab but not etanercept induces apoptosis in lamina propria T-lymphocytes from patients with Crohn's disease. Gastroenterology 2003;124:1774-85.
24. ten Hove T, van Montfrans C, Peppelenbosch MP, van Deventer SJ. Infliximab treatment induces apoptosis of lamina propria T lymphocytes in Crohn's disease. Gut 2002;50:206-11.
25. van Deventer SJ. Transmembrane TNF-alpha, induction of apoptosis, and the efficacy of TNF-targeting therapies in Crohn's disease. Gastroenterology 2001;121:1242-6.
26. Sandborn WJ, Hanauer SB, Katz S, et al. Etanercept for active Crohn's disease: a randomized, double-blind, placebo-controlled trial. Gastroenterology 2001;121:1088-94.
27. D'Auria F, Rovere-Querini P, Giazzon M, et al. Accumulation of plasma nucleosomes upon treatment with anti-tumour necrosis factor-alpha antibodies. J Intern Med 2004;255:409-18.
28. Paleolog EM, Hunt M, Elliott MJ, Feldmann M, Maini RN, Woody JN. Deactivation of vascular endothelium by monoclonal anti-tumor necrosis factor alpha antibody in rheumatoid arthritis. Arthritis Rheum 1996;39:1082-91.
29. Taylor PC, Steuer A, Gruber J, et al. Comparison of ultrsonographic assessment of synovitis and joint vascularity with radiographic evaluation in a randomized, placebo-controlled study of infliximab therapy in early rheumatoid arthritis. Arthritis Rheum 2004;50:1107-16.
30. Haringman JJ, Kraan MC, Smeets TJ, Zwinderman KH, Tak PP. Chemokine blockade and chronic inflammatory disease: proof of concept in patients with rheumatoid arthritis. Ann Rheum Dis 2003;62:715-21.
31. Haringman JJ, Ludikhuize J, Tak PP. Chemokines in joint disease: the key to inflammation? Ann Rheum Dis 2004;63:1186-94.

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