Antirheumatic treatment
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The needle and the damage done*

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Evaluation of sublining macrophages by synovial needle biopsy, CD68 immunohistochemistry, and digital image analysis may help to establish evidence based treatments in RA.

Despite decades of research, the aetiology of rheumatoid arthritis (RA) is still unknown. Early theories about its pathogenesis focused on autoantibodies and immune complexes, T cell mediated antigen-specific responses, a T cell independent cytokine network, and aggressive tumour-like behaviour of rheumatoid synovial tissue.[1] Recently, B cell targeting approaches underline again the role of autoantibodies. None of these concepts alone could explain the chronic inflammation and progressive joint destruction characteristic of RA.

However, knowledge about the pathophysiological interplay of lymphocytes, macrophages, and local synovial fibroblasts within the synovial membrane has advanced over recent years. The success of treatments targeting tumour necrosis factor α (TNFα) and interleukin (IL) 1 suggest a key role for the monocyte-macrophage system in the pathophysiology of the disease. This is further supported by the fact, that macrophages are abundant within the rheumatoid synovial tissue.[2] Moreover, both blood monocytes and tissue macrophages are activated and, in addition to IL1 and TNFα, produce various cytokines, chemokines, and metalloproteinases intimately linked to inflammation and joint destruction.[3–5] The significant association between tissue macrophages and radiological progression of the disease[6] also points towards an important pathophysiological function. Last but not least, several groups have demonstrated a remarkable reduction in the number of synovial macrophages after antirheumatic treatment.[7–10] It is very likely that various pathogenetic mechanisms result in a final common pathway reflected by activation of synovial macrophages.

In this issue of the *Annals* Haringman *et al* examine the effect of antirheumatic treatments on tissue macrophages.[11] They combined serial needle biopsies
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with standardised immunohistological digital image analysis before and after treatment either with disease modifying antirheumatic drugs (DMARDs), biological agents, prednisolone, or a specific CCR1 antagonist. They found a highly significant correlation between changes in synovial macrophages and clinical improvement, reflected by the change in the 28 joint count Disease Activity Score (DAS28) after treatment (Pearson correlation 0.874, p<0.01). Most interestingly, the change in sublining macrophages could explain 76% of the variation in the change in the DAS28. This effect was independent of the specific treatment studied.

> "Both conventional and specific antirheumatic treatments appear predominantly to target the sublining macrophages"

This paper is in line with previous work of this and other groups.[7–10] The merit of the current study is the comparative analysis of the main therapeutic concepts in RA with respect to synovial macrophages and in correlation with clinical improvement. The results form a solid basis for evaluating therapeutic efficacy of established antirheumatic treatments in an individual patient. Moreover, the changes of sublining macrophages may serve as a biomarker for evaluating new antirheumatic treatments.

The most prominent findings of this study and important previous data are reviewed briefly.

EFFECT OF DIFFERENT TREATMENTS ON SYNOVIAL MACROPHAGES
Corticosteroids
Corticosteroids act by transcriptional down regulation of IL1, IL6, and TNFα.[12] Moreover, corticosteroids decrease the production of IL8 and monocyte chemoattractant protein 1 (MCP-1),[13] thus interrupting the self perpetuating influx of monocytes into the inflamed synovial tissue. In addition, corticosteroids affect the

balance of membrane bound and soluble TNFα.[14]

At the systemic level, high doses of dexamethasone up regulate IL10 production and down regulate interferon γ, pointing to a shift towards the Th2 cytokine profile.[15]

In a study by Gerlag *et al*, patients with active RA were randomly allocated to receive either oral prednisolone or placebo for 2 weeks.[7] Synovial biopsy specimens were obtained before and after 14 days of treatment. Significant reduction of infiltrating macrophages occurred after treatment. In the current study by Haringman *et al*, patients received 60 mg prednisolone daily for 7 days, followed by 40 mg/day for another 7 days.[11] Again, there was a significant reduction of tissue macrophages (−51%), accompanied by a reduction of DAS28 of −2.15.

Of note, the effect of corticosteroids was comparable to the changes induced by infliximab on the number of infiltrating macrophages.[16]

DMARDs
Antimalarial drugs
Antimalarial drugs such as chloroquine and hydroxychloroquine accumulate within the lysosomes of macrophages, leading to inhibition of phospholipase A_2 and subsequent inhibition of arachidonate production.[4] In vitro, the production of IL1 and TNFα by lipopolysaccharide stimulated macrophages could be inhibited by high doses of antimalarial drugs.[17]

Gold compounds
Like antimalarial drugs, gold compounds accumulate within lysosomes of synovial macrophages[18] and inhibit Fc and C3 receptor expression, oxygen radicals, and production of IL1, IL8, and MCP-11. Moreover, gold compounds inhibit the antigen- and mitogen-induced T cell proliferation and reduce the angiogenic potential of macrophages.[4] [17]

Within the synovial lining, the number of macrophages is strongly reduced by treatment with gold compounds.[8] Hayman and Cox demonstrated that the activity of the osteoclast tartrate resistant acid phosphatase could be inhibited by gold compounds.[19]

Methotrexate (MTX)
The most commonly used DMARD in RA is known to display several anti-monocyte properties such as the reduction of chemotaxis and the increase of soluble TNFα receptor and the IL1 receptor antagonist (IL1Ra).[20] Therefore, the very early initiation of MTX as soon as RA is diagnosed is now strongly recommended to prevent or to at least delay joint destruction.[21]

In the study by Haringman *et al*, 15 DMARD naive patients started treatment with MTX and synovial biopsy specimens were taken before and after 112 days of treatment.[11] These patients had a −41% change of sublining macrophages and a DAS28 reduction of −1.36.

Leflunomide

Leflunomide has various effects on different cells in RA. Thus, it reduces T cell proliferation and inhibits the differentiation of macrophages into osteoclasts.[22] [23] Within cultured synovial macrophages, TNFα and IL1β production could be inhibited by leflunomide.[24]

In a prospective, randomised, double blind clinical trial comparing MTX and leflunomide, both drugs were equally efficient according to ACR20 response criteria. Also, the histological changes of the synovial tissue, such as the reduced number of infiltrating macrophages, the decreased expression of VCAM-1 and ICAM-1, and reduced MMP-1/TIMP-1 ratio, were similar in both groups after 4 weeks of treatment.[25]

Biological agents
Rituximab and etanercept

In a very recent study, Catrina *et al* assessed the influence of rituximab and of etanercept on apoptosis in synovial macrophages.[26] They found a significant decrease in the number of synovial macrophages after 8 weeks of treatment. The number of apoptotic macrophages was very high as determined by TUNEL staining and activated caspase 3 staining. Therefore, they suggested that apoptosis accounts for the smaller number of tissue macrophages after treatment. This finding is in contrast with the results from Smeets *et al*, who found a rapid reduction of synovial macrophages (within 48 hours), but no evidence of apoptosis.[16] These authors suggested that cell migration into the synovium was decreased.

Haringman *et al* treated 20 patients with RA who were receiving stable DMARDs with infliximab (3 mg/kg at day 1 and at day 15).[11] Arthroscopy was performed after 28 days and showed a significant reduction of tissue macrophages, again associated with a reduction of DAS28.

IL1 receptor antagonist

In a randomised clinical trial,[27] 12 patients with RA were treated with human recombinant IL1Ra. The number of infiltrating synovial macrophages was markedly reduced within the group of patients receiving 150 mg/day IL1Ra. In the placebo group the number of infiltrating cells was increased as compared with the initial tissue sample before treatment.

Small molecules
CCR1 antagonist

Chemokines and their receptors play a part in cell migration and inflammation. They represent an interesting target for anti-inflammatory treatment. In a double blind, placebo controlled, phase IIb trial, patients with active RA were treated with an oral CCR1 inhibitor. Synovial biopsy specimens after 2 weeks of treatment showed that infiltrating macrophages were significantly reduced and at the same time clinical improvement was seen.[28]

SURPRISINGLY UNIFORM HISTOLOGICAL PATTERN AFTER DIFFERENT TREATMENTS

In summary, different therapeutic approaches lead to rather uniform histological changes.

The fact that various therapeutic concepts acting through different mechanisms result in one and the same synovial histological pattern is somehow surprising. There was the same significant change of the number of macrophages after rather unspecific treatment with prednisolone or DMARDs and after targeted treatment with biological agents or even with specific molecules.

> "Different therapeutic approaches lead to similar histological changes"

The architecture of synovial tissue is rather complex. Macrophages are present both within the lining and the sublining layer.[29] The functional diversity of these areas, reflected by different activation markers and adhesion molecules, may point towards different contributions to the pathophysiology of the disease. Within the lining layer, CD14+, CD68+, HLA-DR+ macrophages (type A synoviocytes) are in close contact with transformed-appearing fibroblasts (type B synoviocytes). The lining layer is critically involved in cartilage and bone destruction. Macrophages of the sublining layer are found in infiltrates close to CD4+ and CD8+ T cells.

Both conventional and specific antirheumatic treatments appear predominantly to target the sublining macrophages. Thus, Haringman *et al* showed a significant reduction only of the number of sublining macrophages.[11] The number of lining macrophages did not change significantly. In another study, areas remote from the cartilage-pannus junction responded better to treatment with DMARDs than the regions adjacent to the front of the invasion.[30]

This may explain why current treatments can control local and systemic inflammation in many patients, but cannot, as yet, cure the disease.

Several authors also describe a tendency for cell types other than macrophages, such as T cells and plasma cells, to diminish after treatment.[16] However, these changes did not reach statistical significance.

Compared with other synovial cells, macrophages have the advantage of appearing early, within the first weeks after onset of the disease, and they respond quickly to treatment (in contrast with lining hyperplasia and lymphoid infiltrates). They are therefore suited to monitoring disease activity over time.

Of course, it should be kept in mind, that the above mentioned studies focused on local tissue response. Complex effects—for example, of anti-TNFα treatment, such as suppression of other cytokines, interference with the activation of osteoclasts and circulating monocytes, decreased neoangiogenesis, and activated synovial fibroblasts,[31] were not examined by the current study.

IMPLICATIONS FOR CLINICAL PRACTICE

From a more practical point of view, the similar effect of different treatments on synovial histology, which strongly correlates with clinical improvement is advantageous.

Needle arthroscopy is a feasible and safe method for obtaining synovial tissue from the "site of action". It is highly suitable for monitoring the damage occurring in the target tissue. Combined with standardised immunohistochemistry for CD68+ macrophages, this approach appears to be a valuable tool for evaluation of both disease activity and potential efficacy of treatment, independent of the individual therapeutic approach. As shown by Singh *et al*, infiltrates of macrophages are a very early hallmark of active disease.[32] Synovial macrophages are detectable within the first 6 weeks of symptoms, in contrast with other histological changes such as lymphoid aggregates and lining hyperplasia. This appears to reflect the need to start intensive antirheumatic treatment as early as possible to avoid joint destruction. Early evaluation of synovial tissue obtained by needle biopsy may in the future aid in the initial decision making of differential treatment.

Moreover, by serial evaluations of synovial tissue during treatment, nonresponders may be identified much earlier than usual. In particular, for MTX, the most commonly used DMARD, patients are followed up for up to 6 months before a final estimation of the clinical effect can be made. In the study of Haringman *et al* the reduction of the number of tissue macrophages

could be demonstrated after 112 days.[11] Further studies analysing the time course of the histological changes are needed to establish reasonable time points for needle biopsies for the respective treatments.

In addition, sublining macrophages are an appropriate biomarker for evaluating new treatments, especially as they have the advantage of being "resistant" towards placebo effects or expectation bias.[11]

CONCLUSION

The standardised evaluation of sublining macrophages by applying synovial needle biopsy, CD68 immunohistochemistry, and digital image analysis may contribute to the establishment of evidence based treatments in RA.

The broader use of needle biopsies should be emphasised for improved clinical evaluation and better treatment as well as to extend our knowledge about the individual courses of RA.

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