Rheumatoid arthritis: of mice and men: towards the development of innovative therapies for rheumatoid arthritis

Gerlag, D.M.

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Chapter 11

General discussion and summary
This thesis focuses on the development of innovative therapies for the treatment of rheumatoid arthritis (RA).

In Chapter 1, a general introduction to some of the features of the clinical syndrome of RA and the recent developments in the treatment of RA are described. Novel targeted treatments, the so-called biologics, have revolutionized the treatment of RA for many patients. These therapeutics have been discovered by studies aimed at understanding the pathogenetic mechanism of the disease. Research on the pathogenesis of RA identified several key factors involved in the inflammatory process in this disease. One of these factors is TNFalpha, and blocking this factor by means of monoclonal antibodies or a receptor antagonist is an effective strategy for 60-70% of the patients who experience persistent disease activity despite adequate disease-modifying anti-rheumatic drug (DMARD) therapy. Other recently introduced biologic treatment options show on average comparable efficacy. Since not all patients benefit from these new therapies due to primary lack of response, side-effects, or secondary loss of the initial response, there is still an unmet need for new treatments for selected patient groups.

The discovery and developmental process of new therapeutic targets consists of several phases. The preclinical phase involves in vitro and in vivo testing of the effects of the new therapeutic approach. After studying the in vitro effects, animal models for RA, such as collagen-induced arthritis (CIA) in mice and adjuvant arthritis (AA) in rats are often used to test the compound in vivo. Therapeutic efficacy in the clinically manifest phase in these disease models seems to be the best predictor of clinical efficacy in human RA (1). If a compound shows potential clinical efficacy and when there is no safety signal in the preclinical evaluation, the next step is to test the compound in patients with active RA.

In Part I of this thesis, some novel therapeutic targets are discussed, which were examined using various experimental approaches. Both in vitro and in vivo studies were used to provide information about the potential efficacy of these compounds in the treatment of RA.

Chapter 2 describes the effects of a novel T cell-specific inhibitor of nuclear factor (NF)-κB, a pivotal transcription factor regulating an array of pro-inflammatory mediators in various immune-mediated inflammatory diseases like RA (2). After confirmation of the inhibitory effects of this compound, SP100030, on cultured Jurkat cells and other T cell lines, this NF-κB inhibitor was tested in murine CIA. Treatment with SP100030 significantly decreased arthritis severity from onset of clinical signs to the end of the study. The activity of the compound was confirmed by electromobility shift assay (EMSA) on mouse synovial tissue and histologic evaluation demonstrated a trend toward improvement in SP100030-treated animals. It was concluded that SP100030 inhibits NF-κB activation in T cells, resulting in reduced NF-κB-regulated gene expression and amelioration of CIA.
Its selectivity for T cells could theoretically provide potent immunosuppression with less toxicity than other NF-κB inhibitors.

In Chapter 3, a different approach was used to study the effects of NF-κB inhibition in rats with adjuvant arthritis (AA) by inhibiting the activity of inhibitor of nuclear factor κB kinase β (IkB kinase β, or IKKβ), a key regulator of NF-κB activation (3). The first question was whether its constitutive activation could be sufficient to cause arthritis and subsequently we addressed the question whether suppression of IKKβ may lead to clinical improvement. The constitutive active IKKβ gene was introduced into normal ankle joints of Lewis rats by intra-articular injection of an adenoviral construct, leading to inflammation secondary to NF-κB activation. Conversely, inhibition of NF-κB activation by targeting IKKβ activity in ankle joint of rats with AA showed a beneficial effect on arthritis activity. These data suggest that IKKβ plays a key role in synovial inflammation, and that intra-articular gene therapy inhibiting IKKβ activity could represent an attractive strategy for the treatment of chronic arthritis. Since NF-κB not only plays an essential role in signal transduction in inflammatory diseases but also in processes required for normal cellular functioning, interfering with this signalling pathway potentially has serious side effects when administered systemically. One solution to this problem may be the use of local therapy for the administration of targeted small molecules interfering with NF-κB activity, thus increasing tissue specificity and preserving the delicate balance between suppression of inflammation and maintenance of homeostatic function (4).

Another way of interfering with the processes underlying chronic inflammation would be to block the formation of new blood vessels in the synovial tissue. Using this approach, the supply of metabolites and oxygen, as well as the recruitment of new immune cells from the bloodstream into the synovium may be decreased. In addition, the expression of proinflammatory factors produced by activated endothelial cells should decrease after inhibition of neoangiogenesis. In Chapter 4, apoptosis of endothelial cells was induced in order to ameliorate arthritis activity in murine CIA. Mice were injected intravenously with phage expressing an RGD motif, binding selectively to the αvβ3 and αvβ5 integrins. These integrins are expressed on newly formed blood vessels, which are abundant in inflamed synovial tissue, with the theoretical advantage of some tissue specificity of this therapeutic approach. Although systemic treatment with RGD peptides that are selective for αvβ3 reduces angiogenesis presumably by blocking the function of the integrin (5;6), systemic administration of free RGD-4C peptide in the study presented in this thesis had no effect on arthritis. When an RGD peptide that was covalently linked to a pro-apoptotic peptide was used, a significant decrease in clinical signs of arthritis associated with apoptosis induction in synovial blood vessels was found. RGD-4C may target the proapoptotic peptide specifically to areas characterized by neoangiogenesis, minimizing toxicity in this model. Consistent with this notion, no apoptotic cells were
found in tissues other than the inflamed synovium. These results show that targeted apoptosis of synovial neovasculature may suppress arthritis activity and has potential utility in the treatment of inflammatory arthritis. Furthermore, these data support the feasibility of administering systemic agents that home to sites of inflammation as a means of delivering a therapeutic agent. To date it is still unclear if this approach may be effective in humans. Earlier phase II studies on anti-αvβ3 antibody treatment in RA and psoriasis did not show clinical benefit and were ended prematurely (press release Medimmune, August 2004). Treatments directed to vascular endothelial growth factor (VEGF) showed variable results in different experimental animal models of RA. Published data on anti-VEGF treatment in RA are as yet not available, but in some forms of cancer this approach has proven to be clinically effective (7).

When new therapeutic targets have been identified by in vitro and experimental studies in animal models, these compounds ultimately need to be tested in RA patients. To be able to understand the effects of novel treatments, it is important to evaluate the effects on the primary site of inflammation, the synovium. In Part II of this thesis, studies are described focused on the synovial tissue of RA patients. The features of the synovial tissue in RA and other forms of inflammatory arthritis are reviewed in Chapter 5. The synovial tissue in RA shows a dense infiltrate of cells when compared to normal synovium due to accumulation of T cells, B cells, plasma cells, macrophages, dendritic cells, natural killer cells, mast cells, and neutrophils. In addition, there are increased numbers of fibroblast-like synoviocytes. Increased vascularity and adhesion molecule- and chemokine- dependent recruitment of cells from the bloodstream, inappropriate retention, impaired apoptosis, and proliferation may all contribute to the accumulation of inflammatory cells, many of which exhibit an activated phenotype. In this network, cells interact through cell-cell interactions as well as via soluble mediators, including cytokines, chemokines, matrix metalloproteinases (MMPs), and immunoglobulins. The hyperplastic synovium overgrows and invades the underlying cartilage and bone, causing joint destruction and ultimately disability.

Chapter 6 gives an overview of techniques used by rheumatologists to acquire synovial tissue from patients with RA. Minimally invasive arthroscopic biopsy techniques, using small-bore arthroscopes under local anaesthetics, can be performed in an outpatient setting in a safe and well-tolerated way. Advantages of this technique over blind needle biopsy include the possibility to evaluate the synovium macroscopically and to obtain synovial biopsy samples under direct vision. Furthermore, this approach makes it possible to obtain tissue samples in adequate amounts from small as well as clinically uninvolved joints.

Evaluation of synovial tissue biopsies can be used to identify biomarkers for diagnostic and prognostic purposes (8;9). In addition, synovial tissue analysis could be used to investigate the pathological processes underlying the disease and to study the effects of
anti-rheumatic treatment. Sensitive biomarkers associated with clinical efficacy may help to increase our understanding of the mechanism of action of the therapeutic intervention and may be used for selection purposes during the developmental process of novel targeted treatments (10). This approach may be instrumental in the identification of an early therapeutic effect requiring only small numbers of patients, accelerating decisions in phase I/II studies, and enhancing dose selection before large, conventional clinical trials are conducted. Such studies may provide the rationale for phase III trials that are necessary to determine whether the biological effects found in these earlier studies translate into clinically meaningful improvement. The need for the use of biomarkers in early phase clinical trials to evaluate novel therapies in relatively small proof-of-concept studies is becoming increasingly clear from the large number of compounds in the pipeline of pharmaceutical industry, the increasing difficulty including RA patients with active disease in clinical trials due to the success of available treatment, as well as financial and ethical reasons.

The importance of collecting data on the primary site of inflammation, the synovium, to understand the effects of anti-rheumatic treatment is illustrated by the observation that clinical arthritis activity is accompanied by persistent histologic signs of synovitis after treatment with humanized anti-CD52 antibodies or chimeric anti-CD4 antibodies, despite profound depletion of peripheral blood lymphocytes (11;12). Similarly, recent work has shown that B cells may persist in the synovium in some RA patients after treatment with rituximab, in spite of marked depletion of peripheral blood B cells in nearly all patients (13;14).

In earlier studies using serial sampling of the synovial tissue, the effects of successful treatment with various disease modifying anti-rheumatic drugs, such as gold (15), methotrexate (16-18), leflunomide (18), and corticosteroids (19-21) were shown to be associated with decreased mononuclear cell infiltration. Similarly, successful treatment of RA patients with infliximab (22-26), etanercept (27), anakinra (28), and rituximab (13;14;29;30) resulted in reduced synovial inflammation. Evaluation of serial synovial biopsies has also been used to evaluate the effects of experimental compounds for the treatment of RA, including a synthetic retinoid (31), IL-10 (32), anti-CD4 antibodies administered intra-articularly (33), IFNβ (34), a CCR1 antagonist (35), a CCR2 antagonist (36), a C5a receptor blocker (37), and anti-CCL2 antibody treatment (38). These studies show that it is feasible to use examination of serial biopsy samples to monitor the response to treatment, providing information about the effects related to the mechanism of action of the drug and on biomarkers associated with active treatment.

To formally address the question of which feature in RA synovial tissue samples could be used as a biomarker for clinical efficacy in relatively small studies of short duration, a randomized clinical trial was performed. In this study, described in Chapter 7, patients were treated with prednisolone, a known effective drug in the treatment of RA, accord-
ing to the COBRA (Combinatietherapie Bij Reumatoide Artritis) regimen, or placebo for 2 weeks. As a result of this treatment the mean Disease Activity Score (DAS28) was 2.0 units lower after prednisolone therapy compared with placebo and the sublining macrophages were identified as a useful biomarker associated with the clinical response to corticosteroids. Next, the utility of macrophages in the synovial sublining as a candidate biomarker was tested across discrete interventions and kinetics (Chapter 8). A strong correlation between the mean change in the disease activity score (delta DAS28) and the mean change in the number of sublining macrophages was observed. The change in sublining macrophages could explain 76% of the variation in the change in DAS28 (P < 0.02). The sensitivity to change of the biomarker was high in actively treated patients (the standardized response mean (SRM), a measure of the sensitivity to change, was > 0.8) while the ability to detect changes in placebo treated patients was weak (SRM < 0.3), suggesting that changes in synovial sublining macrophages can be used to predict possible efficacy of anti-rheumatic treatment.

For a biomarker to pass the “discrimination criterion” of the so-called OMERACT (Outcome Measures in Rheumatology Clinical Trials) filter, not only should it exhibit high sensitivity to change, but it should also distinguish between effective and ineffective treatment. Therefore, the data from two recently performed randomized, controlled clinical trials of treatment strategies that were shown to be ineffective in RA were added to the data set described above (39). The weighted mean of the SRM for CD68 positive sublining macrophages was -0.89 (SE 0.12) in the patients receiving effective experimental treatment, 0.20 (SE 0.18) in the patients receiving ineffective experimental treatment, and 0.11 (SE 0.18) in the patients receiving placebo. The difference in the weighted mean of the SRM between the effective treatment group and the ineffective treatment group was significant for CD68 positive sublining macrophages. Hence, a clear distinction between effective and ineffective treatment could be made. Linear regression analysis showed that the mean change in the number of sublining macrophages could predict 80% of the variance in the mean change in DAS28 in each study group. As expected, in contrast to the results obtained using the synovial biomarker, the DAS28 was susceptible to placebo effects, as shown by the weighted mean of the SRM of -0.30 (SE 0.18) in the group receiving ineffective treatment.

Taken together, these studies suggest that serial synovial biopsy can be used for selection purposes during early drug development. In proof-of-concept studies based on this approach three types of data are obtained: 1. clinical data, 2. synovial biomarkers specifically related to the mechanism of action of the therapy, and 3. quantification of the number of CD68 positive macrophages. When there is no change in DAS28, no specific effects related to the mechanism of action, and no change in macrophage numbers after treatment, the drug might not hit the target effectively or the concept behind the role of the target in the pathogenesis might be wrong. A rethinking of the therapeutic
strategy to large clinical trials would then be recommended. It can be anticipated that future development will include the use of more extensive markers of joint degradation -- in addition to the available markers of inflammation -- as well as the use of panels of biomarkers in synovial tissue samples.

To investigate if other molecular techniques applied on synovial tissue would provide additional biomarkers, changes in expression of genes determined by Reverse Transcriptase Quantitative Polymerase Chain reaction (Q-PCR) were studied in Chapter 9. The expression of IL-8 and MMP-1 mRNA was significantly decreased in corticosteroid treated patients compared with placebo, while there was also a trend towards decreased IL-1β and TNF-α gene expression in the prednisolone treated group. Since the change in expression of IL-8 and MMP-1 mRNA could differentiate between effective and ineffective treatment, it was shown that Q-PCR may provide additional biomarkers in proof-of-concept studies to supplement immunohistochemical data.

To study the mechanism of action of prednisolone therapy in RA in more detail, Chapter 10 focuses on the mechanisms by which corticosteroids may decrease synovial cellularity. The decreased cell infiltrate after prednisolone therapy might theoretically result from the induction of apoptosis. However, in vitro studies on peripheral blood mononuclear cells as well as analysis of synovial tissue from patients treated with oral prednisolone showed no evidence of apoptosis induction. Consistent with these results, there was no increase in the serum levels of nucleosomes after prednisolone therapy. The cells may leave the synovial compartment via the lymphatic system and it is conceivable that prednisolone therapy exerts its effects in part by increasing the number of lymphatic vessels in the synovium, similar to observations after anti-TNF therapy (40). Analysis of Lyve-1 expression to detect lymphatic vessels in the synovium, however, did not demonstrate an increase in the number of lymphatic vessels after prednisolone therapy, although the number of paired tissue samples tested was probably too low to draw definite conclusions. We also examined the effects of prednisolone treatment on the expression of adhesion molecules, since these molecules are intimately involved in the migration and retention of inflammatory cells into the inflamed synovial tissue. The use of oral prednisolone lead to down regulation of the expression of adhesion molecules, confirming earlier reports (41;42). Thus, prednisolone therapy appears to reduce synovial inflammation primarily by interfering with migration and retention of inflammatory cells rather than by apoptosis induction, similar to recent findings after TNF blockade (43).

Taken together, studies of rheumatoid synovium contribute to an understanding of the events that take place in vivo and complement experimental animal studies and in vitro studies. Examination of molecular markers in synovial tissue combined with analysis of peripheral blood and synovial fluid is increasingly used in clinical trials on anti-rheumatic
therapies (10). It can be anticipated that examination of synovial biopsy samples will also be developed to predict and monitor the response in individual patients. To facilitate dissemination of this approach, synovial biopsy will probably increasingly be used by means of office-based mini-invasive ultrasound-guided techniques (44).
**Reference List**


