Fatal attraction: chemokines and rheumatoid arthritis
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Chapter 9

GENERAL DISCUSSION AND SUMMARY
This thesis focuses on the feasibility of targeted therapies directed against chemokines and chemokine receptors for the treatment of patients with rheumatoid arthritis (RA) as well as on the understanding of the changes in the synovial tissue (ST) alongside clinical response.

BACKGROUND
During the past 10 years improved understanding of the pathophysiology of RA has led to several key changes in the approach to therapy. It was recognized that early diagnosis and treatment favored disease outcome. In addition, the use of methotrexate and combinations of disease-modifying anti-rheumatic drugs (DMARDs) proved to be effective. Finally, the use of targeted therapies, directed against key-players in the inflammatory process like tumor necrosis factor-alpha (TNF-α) proved to be highly effective in reducing disease symptoms and destruction (1).

The latter development initiated a completely new way of thinking about the treatment of many chronic inflammatory disorders and revolutionized treatment of RA. The fact that not all patients respond and (severe) side effects may occur, encouraged the search for other key players in the inflammatory network, which may be targeted. The discovery of chemotactic cytokines (chemokines) and their receptors in the early 1990’s, was a welcome contribution within this context.

An important contribution to the early chemokine-research came from the HIV (Human Immunodeficiency Virus) field since, in addition to CD4, the 7-transmembrane (7TM) chemokine receptors CCR5 and CXCR4 were identified as co-receptors for HIV transmission (2). Homozygous individuals carrying the A32 mutation (a 32-base pair deletion in the region encoding the second extracellular loop) are largely resistant to HIV infection and small molecule chemokine receptor antagonists may robustly inhibit the infection of human peripheral blood mononuclear cells (PBMCs) in vitro (3). Clinical trials investigating the potential of this approach in humans infected with HIV are in progress (4).

As many chemokines and chemokine receptors are involved in the migration of cells towards the site of inflammation (chemotaxis), angiogenesis, integrin activation and in the stimulation of the release of (pro-)inflammatory cytokines, it is not surprising that this raised interest of researchers in many different fields. In combination with the fact that it is relatively easy to develop small, orally bioavailable molecules against these 7TM receptors, as they already exist in the treatment of ulcers, migraine and allergies, the chemokine family represents an attractive target for drug development.

MAIN FINDINGS
Chapter 2 provides a systemic overview of the literature on chemokines and chemokine receptors in RA. Chemokines form a large superfamily of small (8-14 kDa) cytokines that play a crucial role in cell migration. They interact with G-protein-coupled receptors, possessing a 7TM domain. To date about 50 chemokines have been identified signaling through some 20 distinct receptors (5).

Chemokines are involved in both physiologic and inflammatory processes and many of these molecules are reported to be expressed at higher levels in peripheral blood, synovial fluid (SF) and ST of RA patients compared to controls. In combination with the evidence that cell migration can be effectively blocked in vitro and that chemokine blockade in animal models can be protective against arthritis, the search for suitable candidates for targeted therapy in RA patients is highly justified.

In Chapter 3 the expression of chemokines and chemokine receptors in paired ST and peripheral blood (PB) from patients with RA, osteoarthritis (OA) and reactive arthritis (ReA) was investigated in order to further clarify the expression and discuss the possible function of
certain chemokines and receptors in arthritic disorders. In addition, these studies may assist in the identification of suitable targets for therapeutic intervention.

This study showed that although other receptors and ligands are involved as well, blockade of especially CCR1 and CCR5 may be a potentially effective treatment for a variety of arthritides.

In Chapter 4 the reliability of digital image analysis as a tool for the analysis of ST in randomized controlled trials was investigated. The major advantage of digital image analysis is the standardization of image acquisition and processing, minimizing variance, and the ability to quantify the actual stained area together with staining intensity in a time efficient way (6;7). As the analysis of ST is increasingly used in the evaluation of new targeted therapies in RA patients, reliable and validated methods for studying the ST are pivotal. The study showed that there was high reliability using digital image analysis in the quantification of synovial markers in small proof of principle clinical trials.

In order to further elaborate the changes of ST alongside clinical response, the study presented in Chapter 5 investigated the effects of a known clinically effective treatment (oral prednisolone) on the features of rheumatoid ST. The results showed that effective therapy was particularly associated with a marked reduction in macrophage infiltration in the ST of RA patients after 2 weeks of treatment. Therefore, the change in synovial sublining macrophages may be used as a biomarker when novel therapeutic agents for RA are screened for potential efficacy.

In Chapter 6 the utility of CD68+ macrophages in the sublining layer as a candidate biomarker was tested further across discrete interventions and kinetics. In this study the changes in this biomarker after different therapies and after different time intervals in relationship to the clinical response to treatment were evaluated, to validate the analysis of synovial macrophages in clinical studies. The study showed that there was a high sensitivity to change for synovial sublining macrophages across the different treatment regimens. In addition, the study suggested that after placebo treatment the biological marker may be less susceptible to placebo effects or expectation bias than clinical evaluation. Therefore, the analysis of ST may, in addition to providing insight into the mechanism of action of treatment, help to screen for possible efficacy in small proof of principle clinical trials.

In Chapter 7 the effects of blockade of CCR1 with a small, oral bioavailable molecule in patients with RA was investigated. Although only 16 patients could be evaluated in this randomized controlled trial, ST analysis showed a marked change in the patients treated with the CCR1 antagonist, after only two weeks of treatment. There was especially a large decrease in the number of synovial macrophages, cells that are known to express CCR1 and which are associated with clinical response. Cells that are not capable of expressing CCR1 were not affected by the treatment. The study suggested that blockade of a chemokine receptor in patients with RA is feasible, safe and, at least on a biological level, effective.

Chapter 8 presents a randomized controlled, dose escalation trial with a human monoclonal antibody against CCL2/MCP-1. CCL2/MCP-1 is an important mediator of the migration of monocytes/macrophages through its receptor CCR2. CCL2/MCP-1 is therefore regarded as a possible target in the treatment of RA. Forty-five patients were treated in a double blind placebo controlled manner and evaluated for safety and efficacy. Although the treatment was generally well tolerated, it did not result in detectable beneficial clinical or immunohistologic effects. On the contrary, there appeared to be a dose dependent increase in serum total
CCL2/MCP-1, which seemed related to an increase in serum CRP and ST macrophages in the highest dose group. Thus, the effects of the anti-CCL2 antibody did not induce clinical improvement, which might be explained by immune complex formation.

CONCLUSION AND FUTURE RESEARCH
This thesis investigated suitable targets in the chemokine family for potential treatment of RA. Whether digital image analysis is a reliable tool to study the effects of treatment within ST, which ST biomarkers are most useful in the evaluation of new therapies and if blockade of CCR1 and CCL2/MCP-1 might be a safe and effective in the treatment of RA. The results indicate that it is still difficult to select the best targets for interfering with the chemokine system. Preclinical research is limited by many of the characteristics of the chemokine family. As in vitro redundancy complicates the identification of the best targets, animal studies are needed to confirm the efficacy of compounds in vivo. Animal models may not reproduce all the features of human RA, which hampers the comparison of these models with patients. In addition, species specificity of chemokines and chemokine receptors complicates the use of animal models (8;9).

Nevertheless, it is clear that chemokine receptors like CCR1 and CCR5 are among the best candidates to target, although other chemokines and receptors are involved as well. In light of the need to screen various compounds for potential efficacy in small numbers of patients and because of recent technical developments, our thinking about clinical trials is changing. Clinical studies during early phases of drug development will increasingly consist of small trials with a high density of biological data (10).

Consistent with this notion, serial synovial tissue analysis with the evaluation of biomarkers was recently included in several randomized clinical trials of both DMARDs and biologic agents (4;11-14). These and other studies showed consistent relationships between the magnitude of synovial changes and clinical response. Especially the change in infiltrating sublining macrophages was identified to be a potent and sensitive synovial biomarker. Inclusion of such biomarkers in an early stage of drug development may help to show proof of principle and will facilitate the screening for potentially effective treatment. Obviously, reliable and validated tools for analysis are pivotal. Digital image analysis proved to be a highly reliable method to analyze ST, with regard to intra- and interobserver reliability. The fact that an increasing number of research centers is using this approach warrants further studies comparing the results obtained in different centers.

There are still many questions to be answered regarding the in vivo activity of many of the chemokines and chemokine receptors. Despite the fact that chemokines and chemokine receptors are attributed mainly pro-inflammatory capacities there is also proof that some of them may have regulatory or even anti-inflammatory functions (15;16). In addition, it is possible that some ligands may act as agonists at one receptor and as antagonists at others or are converted in vivo from being agonists into being potent antagonists (17;18). In combination with the possible redundancy it may, therefore, be necessary for some pathways to use poly-chemokine antagonists (19) or a combination of different chemokine antagonists. The identification of the best targets among chemokines/chemokine receptors will be the subject of future research. Therefore, initial small proof of principle studies with a high density of biological data, followed by well-controlled studies of sufficient duration will be essential to appreciate the potential of directly antagonizing leukocyte migration for the treatment of chronic inflammatory disorders.
Reference List


