Anti-TNF therapy in rheumatoid arthritis: Searching for mechanisms of effect
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General Introduction
RHEUMATOID ARTHRITIS

Rheumatoid arthritis (RA) is a chronic systemic disease characterized by synovitis, and progressive damage to cartilage and subchondral bone in the majority of patients, affecting 0.5–1.0% of adults in developed countries. The disease is three times more frequent in women than men and prevalence rises with age and is highest in women older than 65 years. Patients present with symptoms of pain, joint swelling, stiffness and limited range of motion and despite major improvements in anti-rheumatic therapies RA is still associated with increased morbidity and mortality. RA is considered an auto-immune disease, in part because of the presence of auto-antibodies, such as IgM-rheumatoid factor and anti-citrullinated protein antibodies (ACPA), of which especially ACPA are highly specific for RA. They can be found in 70-80% of patients even before the onset of clinical symptoms and their presence is associated with a more severe and destructive disease phenotype. This finding, in combination with improved treatment possibilities and the recognition that early therapeutic intervention improves clinical outcome prompted new criteria for classification. Nowadays, patients are classified on the basis of symptoms, physical examination and laboratory results according to the new 2010 RA criteria developed by the American College of Rheumatology and European League Against Rheumatism (EULAR).

Being a systemic disease, RA also has systemic manifestations, such as fatigue, low grade fever, subcutaneous or pulmonary nodules, pleuritis, pericarditis, vasculitis and interstitial pneumonitis. In addition, during the last years it has become evident that RA patients have an increased cardiovascular risk. Traditional cardiovascular risk factors in combination with the inflammatory status in RA patients contribute to the high prevalence of cardiovascular disease in RA and both risk factor management and adequate treatment, reducing inflammation, are recommended.

In addition, RA has major individual and socio-economic consequences. For the individual, co-morbidities, disease activity and functionality are important factors contributing to the impact on health, which is usually summarized by health-related quality of life measures. Furthermore, the disease can lead to loss of work with loss of income as a consequence. For society there are direct (i.e. healthcare) costs, such as costs of drug treatment and inpatient care, including surgery. These direct costs have risen the last decade mostly due to treatment with biological anti-rheumatic drugs. In addition, there are indirect costs for society, involving factors such as worker absences, reduced productivity due to disability, and early retirement. Interestingly, economic analyses, incorporating indirect costs, support the current treatment recommendations of an early start of traditional disease modifying anti-rheumatic drugs (DMARDs), and rapid treatment escalation when there is insufficient response. Of note, in this setting, the considerably more costly biological treatments appear to provide sufficient cost effectiveness.

PATHOGENESIS

Genetic and environmental studies

The etiology of RA is not completely understood. The combination of genetic susceptibility, with environmental factors may lead to the development of RA. So far, genome wide analyses make it clear that immune regulatory factors underlie the disease. For ACPA positive patients, the
association with HLA-DRB1 has been long-established. In addition, also the association with e.g. TRAF1–CS, PTPN22 and CTLA4 point to a role for involvement of the immune system. Genetic risk factors for ACPA-negative disease have been identified as well. Interestingly, they involve a different subset of genes, suggesting different patho-physiological pathways (reviewed in 11).

Findings from studies of gene–environment interactions complement these observations. Smoking is the best established environmental factor in the pathogenesis of RA, but only in ACPA positive RA12. Data from genetic epidemiological studies have shown that HLA-DR allele positivity and smoking contribute independently to a higher risk of ACPA positive RA12, 13. Smoking may promote post-translational modifications, through peptidyl arginine deiminase, type IV (PADI4), resulting in citrullination of mucosal proteins and loss of tolerance to neoepitopes that can subsequently elicit an ACPA response. Of interest, RA appears to be associated with periodontal disease. *Porphyromonas gingivalis* expresses PADI4, which is capable of promoting citrullination of mammalian proteins14. Other mechanisms may be involved as well in the formation of ACPA in periodontal disease.

Interestingly, both smoking body and mass index (BMI) are significantly associated with the development of arthritis in auto-antibody positive individuals and the combination of smoking and obesity is synergistic15. White adipose tissue is recognized to be a multifactorial organ that can secrete pro-inflammatory factors such as tumor necrosis factor (TNF), interleukin-6 (IL-6), factors of the complement system, growth factors, adhesion molecules and several pro-inflammatory peptides, collectively called “adipo(cyto)kines”. Adipokines were given their name as they were thought to be predominantly produced by adipose tissue. However, it is now becoming clear that they can also be synthesized in other tissues including cells of the hyperplastic synovium16, 17. Adipokines are increased in obesity and appear to contribute to the so-called “low-grade inflammatory state” of obese subjects that can eventually lead to insulin resistance and cardiovascular complications18–20. In RA they have also been suggested to play a role in the increased risk of CV disease, though their biological role is not entirely understood at this point 21. In addition, adipokines represent a new family of compounds that are involved in the modulation of inflammation and the innate immune system and as such might be involved in RA pathogenesis22–24. Supporting the notion that fat tissue may play a role in RA pathophysiology, obesity has been consistently associated with reduced radiological damage in RA25–27, but also to a diminished clinical response to anti-TNF treatment with infliximab in established RA patients28. Both the classical (adiponectin, resistin, leptin and visfatin) and the more recently discovered adipokines (e.g. chemerin and vaspin) have been studied in RA. Several clinical and experimental lines of evidence showed their contributions to rheumatic disorders and their relationship to cardiovascular disease, inflammation and radiological damage, but some data are conflicting and their exact role is not entirely clear at the moment 21, 23.

**Synovial inflammation**

Normally, the synovial tissue consists of an intimal lining layer, comprising a few layers of fibroblast-like synoviocytes, above a loose tissue, called the synovial sublining layer, which consists of a network of collagen fibers and scattered fibroblasts and blood vessels. In RA the synovial tissue mass is characterized by intimal layer hyperplasia due to proliferation and impaired apoptosis of the fibroblasts, which have an altered activation state, and accumulation of intimal macrophages.
The hyperplastic synovial tissue can invade adjacent cartilage and bone at the site of inflammation and is also called pannus at the site of invasion. In the synovial sublining, new vessels are formed that allow influx of inflammatory cells such as B cells, T cells, macrophages, plasma cells, mast cells and dendritic cells. These cells secrete diverse chemokines, cytokines and other inflammatory mediators, which play a role in the migration and retention of these cells in the synovium.

Supported by the finding that inflamed synovium has highly invasive potential it was thought that RA pathogenesis started with inflammation of the synovial membrane, which later spreads over to adjacent structures resulting in penetration into the bone marrow. However, a fundamentally different viewpoint on the pathogenesis of arthritis can also be postulated. Pathological changes may start in the bone marrow space and pro-inflammatory cells may migrate to the synovium, a notion supported by recent MRI studies showing bone cysts and osteitis before synovitis develops (reviewed in [134]). Changes in the lymph nodes with subsequent local ACPA formation could also precede the development of synovial inflammation in ACPA positive RA.

HETEROGENEITY OF RA
RA has been proposed to be a clinical syndrome comprising various disease subsets rather than one pathogenic entity, in which several different pathogenetic pathways are active that all lead to common signs and symptoms. On the clinical level, there is variability in outcome: some patients will have self-limiting disease while others develop persistent disease with consequent joint damage and even extra-articular disease. In addition, the variable response to different targeted therapies suggests that the role of immunological mediators differs between patients. On the biological level, different immunological features can be observed in peripheral blood and the synovium. The majority of patients are auto-antibody positive, but not all; these subsets differ in their association with genotype and environmental factors like smoking, disease course and response to treatment. In addition, gene expression profiling of peripheral blood and synovial tissue also differ between subgroups of RA despite a common clinical presentation. Furthermore, some patients have diffuse lymphocyte infiltration in the synovium, while in others germinal center like structures can be found.

The identification of biomarkers that could reliably distinguish between different subsets of RA might help to predict outcome and response to treatment in individual patients. At this point however, there are no definitive clinical or biological biomarkers that define discrete disease subgroups with very distinct prognostic and therapeutic significance. Future work should address this in more detail, including use of novel technologies and assessment of combinations of clinical, imaging and molecular biomarkers.

TREATMENT OF RHEUMATOID ARTHRITIS
During the past decades important breakthroughs have been made in the treatment of RA. First, the use of low-dose methotrexate and other DMARDs was introduced as effective treatment. Second, the so-called biological therapies revolutionized treatment in RA. These
drugs consist of monoclonal antibodies or receptor constructs and are designed to specifically target one pro-inflammatory molecule or cell type, thereby reducing inflammation. Third, it was recognized that patients may benefit substantially from start of treatment in early disease and prompt adjustment aiming for disease remission.

Methotrexate is considered the anchor drug in RA, as it is reasonably well-tolerated and effective. When the treatment goal is not reached with methotrexate alone, it can be combined with other DMARDs like sulphasalazine, leflunomide or hydroxychloroquine or with a biological agent. Furthermore, there is robust evidence that glucocorticosteroids are effective as bridging therapy. Glucocorticosteroids have been shown to have not only anti-inflammatory but clearly also disease-modifying properties. However, their toxicity particularly in the intermediate to long term, should not be disregarded and thus glucocorticosteroids should be used with caution and preferably for only limited periods of time.

At this point there are 5 classes of biological drugs that have been registered for the treatment of RA. 1. TNF-antagonists (infliximab, adalimumab, etanercept, golimumab and certolizumab) 2. An IL-6 receptor antibody (tocelizumab) 3. A fusion protein consisting of the extracellular domain of human CTLA-4 connected to the Fc tail of human IgG1 (abatacept). This outcompetes the binding of CD28 on a T cell with CD80 and CD86, though its’ exact working mechanism is as yet not completely understood. 4. An anti CD-20 directed B cell depletive antibody, rituximab and 5. An IL-1 receptor antagonist (anakinra). As anakinra is less effective in comparison with the other 4 classes of biologicals, it is less frequently used. TNF-antagonists are currently often used as first line biological treatment in light of the vast experience with these drugs. However, in the Netherlands tocelizumab and abatacept have also been registered for RA in patients who have failed conventional DMARDs. If treatment with TNF-antagonists is not effective, patients are switched to another TNF-blocking agent or another drug.

In daily practice, response to treatment is assessed by using for instance the disease activity scores evaluated in 28 or 44 joints (DAS28 or DAS44): a reduction in DAS28 ≥ 1.2 over time is used as a criterion for response. In clinical studies response is often reported based on the EULAR response criteria (good, moderate or non-response) or ACR response criteria (20%, 50% or 70%). Interestingly, on the group level all currently registered biologicals show similar clinical responses: ACR20 responses of around 70% in MTX inadequate responders and around 50% in TNF inadequate responders. Of note, the majority of the patients do not achieve disease remission. Thus, there is still a clear unmet need.

TNF ANTAGONISTS

Disease control has been greatly improved by the use of TNF blockade in patients with RA and other immune-mediated inflammatory diseases. As described above, five TNF antagonists can be used in clinical practice for RA: the monoclonal antibodies infliximab, adalimumab, golimumab and certolizumab and etanercept, which is a TNF-receptor Fc-fusion protein. The mechanisms by which TNF antagonists exert their effect are still not completely understood. On the synovial level, TNF antibody treatment has been shown to result in marked reduction of...
synovial inflammation as early as 48 hours, 2 weeks and 4 weeks after the start of treatment\textsuperscript{53-54}. Synovial tissue is a dynamic tissue in which the number of infiltrating cells is dependent on the balance between their influx and efflux, proliferation and apoptosis\textsuperscript{55}. Interestingly, TNF blockade was shown not to induce apoptosis in the synovium early after initiation of treatment\textsuperscript{51, 54}, although we cannot exclude the possibility that more long-term treatment would lead to a more pro-apoptotic state, being a result of dampening of inflammation rather than its cause\textsuperscript{50}. Therefore, reduced synovial cell counts after TNF antagonist treatment might be explained by modulation of cell migration. Influx could be reduced by the effect of TNF blockade on several cytokines, chemokines and adhesion molecules, thereby interfering with the interaction between inflammatory and endothelial cells, tissue cells and extracellular matrix\textsuperscript{52, 53}. It is at present not clear if endothelial deactivation is a direct effect of TNF blockade or whether it indirectly results from the decrease in macrophages numbers or expression of cytokines produced by inflammatory cells. Since TNF is known to promote angiogenesis\textsuperscript{56}, TNF blockade might be capable of inhibiting angiogenesis in patients with RA similar to what has been observed in patients with psoriatic arthritis\textsuperscript{57}. Infliximab treatment was indeed associated with reduced VEGF levels in RA FLS in vitro\textsuperscript{58}. Finally, anti-TNF therapy could contribute to the increased egress of immune cells from the synovial membrane by increasing their traffic into the lymphatic draining system. In line with this hypothesis lymphatic vessels are abundantly present in patients with RA and their formation is increased after infliximab treatment\textsuperscript{59}.

Anti-TNF therapy has well-established beneficial effects on disease activity, joint damage and CV disease, though the exact mechanism by which TNF blockade could reduce CV risk remains to be elucidated\textsuperscript{60}. However, 30-40\% of the patients do not respond and patients who exhibit an initial response may lose response due to formation of anti-drug antibodies\textsuperscript{61, 62} or as result of escape mechanisms due to the disease process. In addition, side effects may occur\textsuperscript{63}. Together with the costs associated with biological treatment\textsuperscript{9} there is clearly a strong need to identify predictors of response prior to the start the TNF inhibition as a way to improve cost-effectiveness. Several studies aimed at identification of the predictors of response have looked into demographic, clinical and radiological parameters; blood biomarkers; genetic markers and synovial markers (reviewed in\textsuperscript{54}). However, at this point these associations are not strong enough to predict the response to TNF blockade in the individual patient and this remains an active area of research.

**OUTLINE OF THIS THESIS**

This thesis aims to improve our knowledge of different aspects of TNF blockade in RA by studies of its mechanism of action, predictors of response and the effects on quality of life and ability to work.

Previously, we showed continuous migration of monocytes into the inflamed synovial tissue of RA patients at a slow macrophage-replacement rate\textsuperscript{55}, suggesting that the rapid effect of anti-TNF therapy on macrophage infiltration cannot merely be explained by blockade of cell influx. In chapter 2 we investigated for the first time the effect of anti-TNF therapy on macrophage infiltration into the synovium, using a novel imaging technique. In chapter 3, we
studied the changes in the T-cell repertoire shortly after initiation of infliximab treatment. In addition, we further explored the concept of cellular egress from the synovium as a result of anti-TNF therapy. Recently, we developed a new technique that could provide an accurate quantitative data on the degree of expansion of individual T cell clones within the complete T-cell receptor (TCR) repertoire, allowing quantitative comparisons of clonality between different patients and between different compartments of the body.\textsuperscript{65, 66} Using this technique, we studied the effect of infliximab treatment on the clonal expansion of T-cells. Furthermore, we tested if T cell clones specific for the synovium (i.e. not detectable in peripheral blood at the start of the study), that decrease from the synovium after treatment with infliximab therapy can be found in the peripheral blood 48 hours after treatment.

In chapter 4, we studied if synovial CD11c expression at baseline is associated with the clinical response to infliximab treatment. Previous data suggested a role for CD11c in the migration and/ or retention of macrophages in synovial tissue\textsuperscript{67} and expression of CD11c on peripheral blood CD14+ monocytes has been found to be significantly upregulated at baseline in responders to adalimumab therapy as compared to non-responders or healthy controls, although this was only observed in patients who did not use methotrexate co-medication\textsuperscript{68}. Taken together, these data could make CD11c an interesting candidate biomarker to refine the current prediction model based on synovial biomarkers.

Obesity is associated with a diminished clinical response to anti-TNF treatment with infliximab in established RA patients and adipose tissue itself may play a role in creating a more therapy resistant state\textsuperscript{28}. Therefore, we investigated the role of anti-TNF therapy on adipokines as they are increased in RA and may exert pro-inflammatory and destructive effects\textsuperscript{23}. In chapter 5, we investigated the effects of 16 weeks of anti-TNF treatment on adiponectin, resistin, leptin, visfatin and vaspin levels and compared the results with the effects of glucocorticosteroids. We related these effects to clinical parameters like, disease activity, radiological damage and lipid index. In chapter 6, we investigated the effect of anti-TNF treatment of chemerin, a novel adipocytokine and chemokine involved in the recruitment of dendritic cells and macrophages.

Finally, in chapter 7, we studied the effect of anti-TNF treatment on workability, fatigue and quality of life. After all, these outcomes are of great importance to patients as they directly influence their daily life.

**REFERENCE LIST**


