B cells and B cell directed therapies in rheumatoid arthritis: towards personalized medicine
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CHAPTER

GENERAL INTRODUCTION
INTRODUCTION

RHEUMATOID ARTHRITIS (RA) is a chronic inflammatory condition of unknown origin which affects around 1% of the population worldwide. Patients experience swelling, pain and limited motion of joints due to inflammation of the synovial tissue lining the inside of joints. Characteristically, RA manifests itself as a symmetric polyarthritis that involves the metacarpophalangeal joints. Some patients may experience a mild illness, but the majority of patients suffer from an invalidating condition that during its course leads to development of joint destruction, progressive invalidity and associated morbidity and mortality.

In this thesis we investigate B cells, key players in the pathogenesis of RA, and B cell directed therapy to further improve the treatment of RA.

DEVELOPMENTS IN THE DIAGNOSIS OF RA There is no specific diagnostic test to differentiate RA from other types of arthritis. However, around 70-80% of patients have elevated serum levels of rheumatoid factor (RF), autoantibodies directed against antibodies of the IgG class. The classification criteria for RA, designed for epidemiological studies, were first defined in 1958 and revised in 1987. They include the presence of a symmetric polyarthritis and/or rheumatoid factor, extra-articular manifestations and bone erosions.

In the 1990s it was found that around 70-80% of RA patients also have antibodies against citrullinated peptides. The presence of anti-citrullinated peptide antibodies (ACPA) is more specific for RA than the presence of RF. ACPA and RF were also shown to be present before the onset of manifest arthritis. The presence of ACPA has therefore been incorporated in the recently revised diagnostic criteria for RA. These criteria have been adapted to include early markers of disease activity.

DEVELOPMENTS IN THE TREATMENT OF RHEUMATOID ARTHRITIS During the last fifteen years the treatment of RA has markedly improved. First, as mentioned above, better diagnostic markers have been developed, resulting in recognition of the disease in an earlier stage. Second, RA is treated more aggressively. Disease-modifying antirheumatic drugs (DMARDs), especially methotrexate, have replaced non-steroidal anti-inflammatory drugs (NSAIDs) as first-line treatment. Third, increasing knowledge of the underlying pathogenetic process has resulted in a growing armoury of new treatments. These new, targeted, treatments have supplemented and in part replaced conventional DMARDs. They have been designed using a biotechnological approach and are therefore called ‘biologics’. The first biologicals registered for RA block the function of the cytokine TNF, which is abundantly present in the synovial tissue of RA patients.

These TNF blockers are infliximab (a chimeric antibody), adalimumab (a humanized antibody) and etanercept (a soluble receptor). More recently, certolizumab (a pegylated antibody fragment) and golimumab (a fully human monoclonal antibody) were registered. In patients who fail initial treatment with methotrexate or other DMARDs, treatment with a combination of a TNF blocker and methotrexate is effective in a subset of RA patients. In randomized controlled trials a 20% improvement in disease activity according to the American College of Rheumatology (ACR) response criteria was found in around 50-80% of the patients, a 50% improvement in 20-50% of patients and a 70% improvement in 10-25% of patients, which was statistically significant when compared to placebo treatment.

Other biologicals that have been registered as treatment for RA are rituximab, which depletes CD20 positive B cells, abatacept, which blocks the interaction between CD80 and CD86 on T cells and antigen presenting cells, and tocilizumab, which blocks the IL-6 receptor. These biologicals induce on average a decrease in disease activity in a similar percentage of patients compared to TNF blockers. Despite the advent of these new treatments early disease remission is only achieved in a proportion of patients and patients need to be treated with often relatively expensive treatments. There is therefore a continued need to better understand the disease pathophysiology to further improve treatment of RA.

RA PATHOPHYSIOLOGY The synovial tissue normally consists of an intimal lining layer, comprising a few cell layers of fibroblast-like synoviocytes, above a loose tissue, called the synovial sublining layer, which consists of a network of collagen fibres and scattered fibroblasts and blood vessels. In RA patients the synovial tissue mass is increased due to influx of inflammatory cells and proliferation of synoviocytes. The hyperplastic synovial tissue invades adjacent cartilage and bone, ultimately resulting in joint destruction. The inflammatory cell infiltrate consists of macrophages, mast cells, natural killer cells, dendritic cells, T cells, B cells, plasma cells, and neutrophils. These cells secrete diverse cytokines, chemokines and other inflammatory mediators.
The etiology of RA is currently unknown. A body of evidence indicates that genetic predisposition, environmental factors and immune mechanisms are involved in its pathophysiology\(^6\). The strongest genetic link is that between RA and the presence of a polymorphism in HLA-DRB1, encoding the ‘shared epitope’\(^22\). Recent genome-wide association studies have identified weaker associations between RA and polymorphisms in other risk loci. The causal genetic mutations still have to be determined for the majority of these risk loci. Up till now, genetic data suggest a link between RA, inflammatory pathways and defective antigen presentation. Furthermore, epidemiologic studies have found an association between RA and smoking. Supporting evidence for other environmental risk factors is weak\(^6\). With regard to immunological mechanisms the different inflammatory cells and mediators that are present in the inflamed synovial tissue have shown to play a role in RA pathophysiology\(^\text{22}\).\n
**HETEROGENEITY IN RA PATHOPHYSIOLOGY** Multiple lines of evidence suggest that RA is a shared clinical manifestation of different pathogenetic conditions. On the clinical level this is suggested by the fact that the severity and course of arthritis differ between RA patients and that bone erosions and extra-articular manifestations do not always occur\(^23,24\). Furthermore, certain genetic and environmental factors, such as polymorphisms in HLA-DRB1 and smoking, predispose to RA, but do not occur in all patients\(^22\). On the biological level, different immunological mediators, such as B cells, T cells, macrophages, diverse cytokines and chemokines, have been shown to play a role in RA, but the variable response to targeted treatments suggests that the role of immunological mediators, such as IL6 and TNFα, differs between patients\(^6\). In line with this hypothesis, detailed immunological analyses have shown considerable variability in immune responses between different patients. For instance, the extent and pattern of lymphocyte infiltration in the synovial tissue varies widely between patients. In some patients a diffuse or scarce infiltrate is found, while in other lymphocyte aggregates are found with characteristics resembling those of germinal centers of lymphoid tissue, a process which is called lymphoid neogenesis, and which is caused by a process called lymphoid neogenesis. Furthermore, B cells may have multiple additional potential roles in RA. After B cells are activated they acquire distinct phenotypes. They differentiate either into antibody secreting plasma cells, central memory B cells or one of the effector B cell types\(^23,24\). Effector B cells secrete polarized arrays of cytokines, dependent on the mode in which they are stimulated. Effector B cells can activate T cells and thereby stimulate their proliferation, differentiation and polarization, and enhance/sustain the activation of primed T cells\(^6\). In line with this, T cell activation in the synovial tissue of RA patients is dependent on the presence of B cells\(^6\). Furthermore, B cells belong to the cells that regulate lymphoid tissue architecture and ectopic lymphoid neogenesis which, as mentioned, also occurs in RA synovial tissue\(^6\). Finally, B cells cross-talk with dendritic cells in the process of T cell activation and can acquire a regulatory phenotype\(^6\). It is however unknown whether this is also relevant for RA.

The clinical response to rituximab differs between patients, which suggests that the role of B cells may differ between patients. B cells are important as producers of autoantibodies. RA is related to the presence of diverse autoantibodies. As mentioned, the two most frequently occurring are RF and ACPA, which occur in about 70% of the patients\(^6\). As mentioned above, RF are autoantibodies directed against autologous antibodies of the IgG class. RF were for long regarded as an epiphenomenon, since IgG is a ubiquitous antigen and IgM-RF-IgG complexes are too large to enter the synovial tissue\(^6\). However, recent experimental research suggests that certain RF isotypes are capable of entering the synovial tissue and sustaining RA synovial inflammation\(^6\). Furthermore, it has been shown that RF and other autoantibodies are produced locally in the synovial tissue\(^6\).

ACPA are directed against citrullinated proteins\(^6\). Citrullination is a form of post-translational modification of proteins, in which the amino acid arginine is converted into citrulline. This process occurs amongst others in the inflamed synovium, but also in other inflamed tissues\(^30\). The citrullinated antigens against which ACPA are directed differ between patients. In most cases their target is citrullinated fibrinogen, in others vimentin or α-enolase or type II collagen\(^30\). Present of RF and ACPA has been associated with a history of smoking, polymorphisms in HLA-DRB1, a more aggressive disease course and an improved response to rituximab\(^22\).

**The role of B cells in RA pathophysiology** When focussing on B cells a role for B cells has been proven by the effect of rituximab in RA\(^6\). However,
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Interfering with the humoral response in RA

Rituximab is a B cell depleting antibody that was registered for the treatment of B-cell non Hodgkin lymphoma (B-NHL) in 1998 and in 2006 for RA. Rituximab induces a clinical response in the majority of RA patients, although only a minority displays a robust response to treatment. Rituximab is a chimeric anti-CD20 antibody inducing a temporary depletion of CD20 positive B cells. CD20 is a membrane bound phosphoprotein involved in T cell independent antibody responses. Rituximab induces a rapid, near complete depletion of B cells in peripheral blood. Only B-cell subsets from the immature phase in the bone marrow unto the memory B cells stage are affected, since stem cells, pro-B cells and plasma cells do not express CD20. The depletion lasts for at least four months, after which B cells return in a proportion of patients. The median time of B cell return is 8 months. In vitro rituximab is able to deplete B cells by apoptosis, complement dependent cytotoxicity and antibody dependent cell-mediated cytotoxicity. It is unknown which mechanisms prevail in vivo. Animal models have suggested that rituximab-induced B cell depletion varies among different tissues and that different effector mechanisms may be important for depletion of different B cell subsets. In patients with B cell non-Hodgkin lymphoma (B-NHL) efficacy of rituximab has been related to a polymorphism in the Fc receptor gene, but these data could not be confirmed in other cohorts. It is unknown which effector mechanism prevails in depleting pathogenic B cells when rituximab is administered for RA.

In RA patients, rituximab is currently administered by 2 infusions of 1,000 milligram in 2 weeks time. This represents a simplified non-body surface area based version of the treatment schedule used in B-NHL. Of interest, in B-NHL patients with a large tumor mass, rituximab levels are lower and rituximab is less efficacious. Rituximab could ameliorate disease activity in a number of ways in line with the multiple roles of B cells. First, it could impair the activation of pathogenic T cells. Second, it could interfere with the architecture of lymphoid tissue and/or synovial lymphoid neogenesis. Third, it could inhibit pro-inflammatory cytokine production by effector B cells. Finally, it could block the formation of autoreactive plasma cells. After treatment a slow decrease in RF and ACPA levels is found, larger than the decrease in the total antibody titers and serum titer of antibodies against microbial antigens, such as Streptococcus Pneumoniae and Clostridium Tetani. This suggests that RF and ACPA producing plasma cells are more severely affected by the administration of rituximab than plasma cells producing protective antibodies. This could be a consequence of a shorter life span of autoreactive plasma cells or of the disappearance of inflammatory survival factors after treatment.

Alternatively, one might interfere with the humoral response in RA using atacicept, a fusion molecule of the soluble TACI receptor and IgG. The TACI receptor binds the B-cell associated factors B-Lymphocyte Stimulator (BLyS), A Proliferation-inducing Ligand (APRIL) and the heterodimer of these 2 proteins. BLyS and APRIL are involved in B cell survival, differentiation and class-switching during different stages of B cell development. Both BLyS and APRIL levels are elevated in blood and synovial fluid of RA patients. Mice transgenic for BAFF spontaneously develop autoimmune manifestations. Atacicept treatment could perhaps represent an alternative therapeutic approach in RA. Its application may also increase our understanding about the role of BLyS and APRIL in RA pathogenesis.

Aim and Outline of this Thesis

As a result of the development of new treatments for RA and their application at an early disease stage disease, progressive joint destruction can currently be inhibited in the majority of the patients. Nonetheless, the response to currently registered treatments differs between RA patients and disease remission is only achieved in a proportion of the patients. Furthermore, patients need to be treated chronically with often relatively expensive drugs. There is therefore a need to further improve the treatment of RA. The ultimate goal is to achieve remission in every patient by early intervention based on the treat to target principle. The use of biomarkers may perhaps facilitate the optimal choice of specific therapies in the context of personalised medicine. To achieve this, a number of steps need to be taken: first, biomarkers need to be identified that can predict which patients will benefit most from a specific treatment. Second, we need to understand which immune mechanisms continue to drive the disease in patients do not respond to current therapies. This knowledge can be used to develop novel therapies and to optimize current treatment schedules. Finally, we need to investigate the safety and efficacy of novel treatments.

In this thesis we focus on B cells and B cell-directed therapies. In chapter 2 and 3 we analyse the association between synovial lymphoid neogenesis, clinical and immunological characteristics of RA and clinical response to TNF blockade. In chapter 4 till 7 we investigate the mechanism of action of rituximab in the synovial tissue and peripheral blood in relationship to clinical response. In chapter 8 to 10 we study the current rituximab treatment schedule: in chapter 8 we analyse rituximab levels and formation of anti-rituximab antibodies in relationship to the extent of B cell depletion and the clinical response to rituximab. In chapter 9 and 10 we examine the efficacy of rituximab in a disease activity based schedule, in initial responders versus non-responders to rituximab. In chapter 11 we describe a phase Ib clinical trial to study the safety and efficacy of atacicept for treatment of RA.
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