B cells and B cell directed therapies in rheumatoid arthritis: towards personalized medicine
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CHAPTER 12

B Cells and B Cell-directed therapies in Rheumatoid Arthritis

GENERAL DISCUSSION
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INTRODUCTION

Within the past decade a number of novel effective treatments have become available for rheumatoid arthritis (RA), among which tumor necrosis factor (TNF) blockers, rituximab, abatacept and tocilizumab. Nonetheless, the response to these treatments differs between RA patients and disease remission is only achieved in a proportion of patients and patients need to be treated chronically with often relatively expensive drugs. There is therefore a need to further improve the treatment of RA. This could be achieved by combination of different strategies. First, more optimal use of currently available treatments is needed. The use of biomarkers could play a role in optimising therapy in individual patients. For this purpose, biomarkers need to be identified that patients will benefit most from a specific treatment. We also need to understand which immune mechanisms contribute to the disease in patients who do not respond to current therapies. Subsequently, we can use this knowledge to develop novel therapies and to optimize current treatment schedules.

Multiple lines of evidence suggest RA is a heterogeneous disease with a variable response to targeted therapy. As discussed in chapter 1 this heterogeneity is observed at the clinical, genetic and immunological level. Detailed analysis of immunological mechanisms in RA and assessment of their relationship with genetic and environmental risk factors, clinical characteristics and treatment response may improve our understanding of the pathophysiologic heterogeneity in RA, yield biomarkers to predict response and help to design novel treatments. In this thesis we focussed on B cells and B cell-directed therapies. We investigated whether we could identify biomarkers predictive of the clinical response to TNF blockade and rituximab treatment. Furthermore, we evaluated repeated treatment in initial clinical responders versus non-responders to rituximab. Finally, we performed a phase 1 study with the novel B cell modulating agent atacicept.

SYNOVIAL LYMPHOID NEOGENESIS AS A BIOMARKER FOR RESPONSE TO TNF BLOCKADE IN RA.

The synovial tissue of RA patients is infiltrated predominantly by macrophages and lymphocytes, but also contains dendritic cells, mast cells, natural killer cells and neutrophile granulocytes. When focussing on lymphocytes, the level of infiltration differs between patients. In some patients the tissue contains a diffuse or scarce infiltrate of T cells, while in others B and T cells are organized in lymphocyte aggregates, which are surrounded by fields of plasma cells. Large lymphocyte aggregates may exhibit features of germinal centers of secondary lymphoid tissue, which has been termed synovial lymphoid neogenesis (SLN). This raises the question whether they refine and amplify local humoral autoimmune responses against for instance rheumatoid factor (RF) and anti-citrullinated peptide antibodies (ACPA).

It has been proposed that synovial tissue with SLN forms a pathophysiologic subtype of RA, characterized by amplified local autoantibody production, associated with a more severe, aggressive disease course and differential response to therapy. In line with this hypothesis the presence of SLN was found to be associated with RF positive disease, the presence of erosions and rheumatoid nodules. However these observations were made in small patient cohorts using a selection of patients with end-stage disease. In this thesis we studied whether the presence of SLN can be used as a biomarker to identify clinically and immunological patient subsets and whether, as such, its presence can be used to predict clinical response to treatment.

In chapters 2 and 3 we analyzed the relationship between SLN, humoral autoimmunity, clinical disease characteristics and treatment response in a large and representative cohort of RA patients. First, we analyzed the relationship between SLN and disease characteristics. We found that the presence of SLN is related to increased local and systemic inflammatory parameters, but it is found equally frequent in seropositive and seronegative RA patients. Furthermore, SLN was not associated with increased clinical disease activity parameters or the presence of erosive or nodular disease. We recently confirmed and extended these findings in a prospective cohort of patients with early arthritis. The presence of SLN was neither associated with diagnosis nor with development of persistent or erosive disease. The presence of SLN decreased over time in individual patients, which probably reflected reduced synovial inflammation after anti-inflammatory therapy or self-limiting synovitis in this particular cohort.

The presence of SLN in both seronegative and seropositive RA, as well as other forms of arthritis, suggests that SLN is a phenomenon which is not necessarily associated with autoantibodies. In line with this we recently found that even in seropositive patients the presence of SLN is not associated with an increase in local ACPA or RF production compared to other antibodies. Taken together, despite the fact that SLN has germline-like features, the data imply that SLN is rather an inflammation-driven humoral response phenomenon than a true germline center reaction that refines and amplifies a local autoantibody response. SLN may contribute to inflammation by enhancing T cell activation, promoting local differentiation of plasma cells and by production of cytokines.

In chapter 3 we analyzed whether SLN predicts the response to TNF blockade. We found that patients with SLN overall responded well to TNF blockade and that SLN predicted clinical response independent of other known predictors, such as disease activity at baseline, the presence of ACPA and the level of synovial TNFα expression. Furthermore, we found that SLN is rapidly reversible after treatment. SLN tends to disappear already 48 hours after treatment, before a decrease in DAS28 occurs. These data suggest that TNF is involved in the formation of SLN. In line with this, previous studies showed that TNF blockade results in decreased expression of cytokines, chemokines and adhesion molecules, which are required for secondary lymphoid organ formation.

In summary, we found that SLN does not define a clinically distinct disease phenotype. Furthermore, the data suggest that while SLN exhibits some features of germinal centers it does not function as such. As an inflammation driven humoral immune phenomenon it predicts a good response to infliximab. Nonetheless, the predictive value of SLN as a biomarker of response to TNF blockade in itself is not high enough to be used as a solitary biomarker, indicating the involvement of other, as yet unknown mechanisms. Future work should expand the search for other biomarkers and molecular networks, as well as combinations of clinical variables, to achieve an effective approach that will increase the percentage of patients exhibiting a robust response to TNF blockade.
Mechanism of action of rituximab and biomarkers of response. In chapter 4 to 7 we studied the effects of rituximab on synovial tissue and peripheral blood in relationship to clinical response. We identified biomarkers of response and immune mechanisms that remain active in patients who do not respond to therapy.

Rituximab induces a rapid, near complete depletion of CD20 positive B cells in peripheral blood. In chapter 4 we analysed to which extent rituximab depletes B cells in the synovial tissue of RA patients. We found that rituximab induces a significant decrease in synovial B cells within 4 weeks after administration, but that B cells persist in a proportion of patients. This variable depletion of synovial B cells has been confirmed by other groups. The percentage of patients in whom synovial B cells persist differed between these studies. As discussed in chapter 5 these differences may depend on the time point at which the second synovial biopsy was taken (i.e. at 4, 12 or 16 weeks after treatment) and the choice of the specific antibodies used to detect B cells by immunohistochemistry. Similar to synovial tissue, it was found that B cells, especially a memory phenotype, persisted in bone marrow of RA patients. Furthermore, B cells were found to persist in lymph nodes of patients with B cell non-Hodgkin lymphoma (B-NHL). In our and other studies the persistence of synovial B cells did not predict clinical non-response to rituximab.

Conceivably, the clinical response to rituximab could be improved by achieving more complete depletion of B lineage cells in the tissues. This might be achieved by repeated administration of rituximab in patients with incomplete response, an approach we investigated in chapter 9 (see below). Alternatively, B cell depleting agents might be developed with increased efficacy. A number of these drugs are currently under development. Finally, rituximab could perhaps be combined with therapeutics that enhance B cell depletion. Up to date, no factors have been identified that play a role in protecting B cells from rituximab in synovial tissue. Roles have been suggested for Fc receptor polymorphisms, expression of the complement-inhibitory factors CD55 and CD59 and the B cell survival factors BllyS and CXCL12, but definite prove for a protective effect has yet to be given.

In chapter 6 we analyzed the indirect effects of rituximab on synovial inflammatory cell populations and the differences in these populations between responders and non-responders to treatment. We found that rituximab induces a decrease in synovial T cells and disrupts the presence of SLN and follicular dendritic cells in both responders and non-responders. Furthermore, we found that rituximab induces a decrease in synovial macrophages, especially in the intimal lining layer. Finally, we found an indirect decrease (between 4 and 16 weeks after treatment) in synovial plasma cells in clinical responders. The decrease in plasma cells was correlated with the decrease in ACPA levels in peripheral blood. Of interest, we recently found that the serum levels of free light chains, plasma cell products with a half life of a number of hours, among clinical responders to rituximab. Free light chain levels are not affected by infliximab, indicating a specific indirect effect of rituximab, through depletion of CD20 positive precursors, on short lived plasma cells that produce free light chains. In contrast to observations in clinical responders, we found that synovial plasma cells and serum free light chains persist in non-responders to rituximab treatment. Persistence of synovial B lineage cells in non-responders to rituximab was also observed in other studies. These data suggest that patients may fail to respond to rituximab due to persistence of pathogenic B cells. We did not find baseline differences in the number of synovial B or plasma cells, the levels of RF, ACPA or free light chains in responders versus non-responders. Of note, all patients in our study were selected on the basis of being positive for RF and/or ACPA. Previous work has shown that autoantibody positive RA patients respond better to rituximab treatment than those who are autoantibody negative. Taken together, these data support the notion that autoantibody production by B cell-derived plasma cells plays an important role in promoting synovial inflammation. Conceivably, effectiveness of rituximab treatment may be increased in autoantibody positive non-responders by strategies aimed at suppression of plasma cells associated with autoimmunity in the tissues.

In chapter 7 we studied the relationship between the response to rituximab and the type I interferon (IFN) signature in peripheral blood mononuclear cells (PBMCs). The type I IFN signature is found in a subset of RA patients as the dominant signalling signature in PBMCs. It is also found in a proportion of patients with other autoantibody associated autoimmune diseases, such as Sjogren’s syndrome, multiple sclerosis, dermatomyositis, type 1 diabetes mellitus, systemic sclerosis and systemic lupus erythematosus. Rituximab has shown to be clinically efficacious in many of these conditions. In the two cohorts that we studied rituximab was less effective in RA patients with the IFN signature. These data suggest that the type I IFN signature might be used to predict beforehand which patients will benefit most from rituximab treatment. Still, the predictive value of the type I IFN signature in itself was not sufficient to be used as a solitary biomarker. Therefore, the type I IFN signature should be assessed in future studies in combination with other biomarkers for its validity to guide rituximab treatment decisions.

In chapters 8 to 10 we studied the current treatment schedule of rituximab, which consists of a course of 2 times 1,000 milligram of rituximab. In B-NHL patients rituximab is less efficacious in patients with a large tumor mass and rituximab levels are lower after therapy. In chapter 8 we analyzed whether we could find a similar relation in RA. We found that rituximab levels were not related to the extent of B cell depletion after treatment, or to clinical response. We also found that clinical response was not significantly lower in patients who formed anti-rituximab antibodies. These data indicate that the current rituximab treatment regimen results in drug levels that are in the therapeutic range in both responders and non-responders to treatment, even when patients form anti-rituximab antibodies.

In chapters 9 and 10 we studied the effects of repeated treatment with rituximab in initial responders and non-responders to treatment. In our cohort, initial non-responders did not respond to re-treatment, while clinical responders had an improved response to a second treatment course. Our findings have recently been confirmed in a large, randomized clinical trial. Other studies, however, have suggested that seropositive patients who fail to respond to a first course of rituximab may still respond to a second course. Light of the availability of other therapeutic options, for individual patients who are rituximab non-responders other treatment options should be considered.

Chapter 11 describes the results of a phase Ib trial with atacicept in RA patients. Atacicept is a recombinant fusion protein that binds and neutralizes the activity of the cytokines B lymphocyte stimulator (BlyS) and APRIL. A proliferation-inducing ligand (APRIL) and the heterodimer of these 2 cytokines (BlyS and APRIL) enhance B cell survival, proliferation, antigen presentation, and class-switch recombination at various stages of B cell development. We found that atacicept is well tolerated with an in vivo biologic effect consistent with its mechanism of action. Treatment resulted in a decrease in serum RF and ACPA levels in parallel with total serum immunoglobulin levels. Future work will need to address whether this biological effect might translate into clinical efficacy.
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The persistence of B lineage cells in the synovium of autoantibody positive RA patients who do not exhibit a robust response to the first therapy. The presence of B lineage cells in the synovium of non-responders. Finally, we conducted an exploratory study on the effects of rituximab in RA. The results presented here support the notion that RA is a heterogeneous disease with a variable response to a given therapy and indicate that specific biomarkers may predict the response to therapy. The persistence of B cells and B cell-directed therapies in rheumatoid synovium indicates that novel approaches may be needed to interfere with these cells in initial non-responders.

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Application.

rationale, mechanisms, and clinical
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humans results in impaired T cell-

management and bone marrow of patients with rheumatoid arthritis.

with adalimumab may reverse
gained resistance to rituximab in non-Hodgkin lymphoma.

T-cell differentiation in bone marrow of patients with rheumatoid arthritis.

with anti-CD20 monoclonal
together with adalimumab may reverse acquired resistance to rituximab in non-Hodgkin lymphoma.

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