Targeting the humoral immune system of patients with rheumatoid arthritis

Teng, Y.K.O.

Summary, conclusions and future perspectives

In the present thesis the clinical and immunological effects of targeting the humoral immune system in RA patients is investigated. A general introduction on rheumatoid arthritis (RA) and current perspectives on the role of the humoral immune system are summarized in ***chapter 1***. B-cell depletion is a new strategy for treating patients with rheumatoid arthritis (RA). In the past years, several studies have proven the efficacy of anti-CD20 mediated B-cell depletion with rituximab (Mabthera®) in RA patients who failed TNF-blocking therapy, as summarized in ***chapter 2***. The important role of B-cells in the pathogenesis of RA is deducted from the specific detection of autoantibodies in RA and infiltration of B-cells and plasma cells in inflamed synovium. Even though relatively few RA patients have been treated with rituximab, and therefore long-term data are lacking, the data collected thus far suggest a relatively good safety and efficacy profile. Within the large spectrum of disease modifying anti-rheumatic drugs (DMARDs) and biologicals, such as anti-tumor necrosis factor (anti-TNF), rituximab treatment is at present indicated only for refractory RA patients that have failed at least one TNF-blocking agent.

Chapter 3 demonstrated that rituximab led to the complete depletion of CD20+ B-cells in blood, bone marrow and synovium of the majority of RA patients. However, not all B-lineage cells were completely depleted, as illustrated by the persistence of CD19+ B-cells in bone marrow and CD79a+ B-cells in synovium after treatment. Additionally, rituximab led to significant reductions in serum Ig, which was also reflected in significantly lower titers of ACPA-IgG, ACPA-IgM and RF-IgM autoantibodies. Moreover, we showed that a positive ACPA-IgM status, combined with high synovial B-cell infiltration, predicted a moderate or no response to rituximab treatment.

We then further investigated the relationship between synovial B-cell infiltration, responsiveness to rituximab treatment, and B-cell reconstitution. We previously showed that ACPA-IgM and synovial B-cell infiltration were closely linked in RA patients, irrespective of the presence of CD20+ B-cells, suggesting an important role for ACPA-IgM-producing plasmablasts in the persistence of RA (chapter 3). In keeping with this, we observed that attaining a low disease activity following rituximab treatment was associated with reduced synovial infiltration of CD79a+ CD20- plasma cells, as described in ***chapter 4***. Moreover, a significantly slower repopulation of B-cells was observed in these patients. Altogether, this study demonstrated that rituximab led to low disease

activity in patients who had reduced B-cell proliferation together with reduced infiltration of early plasma cells in synovium.
Moreover, in ***chapter 5***, we demonstrated that of all lymphocyte subsets, only the proportion of B-cells in peripheral blood and bone marrow differed between RA patients and healthy controls, i.e. by being significantly lower. Analysis of B-cell subsets revealed that a reduction of post-switched B-cells was predominantly responsible for the observed differences. In contrast, a higher circulating fraction of phenotypic immunoglobulin-secreting cells (ISCs) was present in RA, the functional relevance of which was underpinned by the association between inflammation and high serum levels of total immunoglobulins and RA-specific autoantibodies. Collectively, these data indicated that RA patients showed enhanced differentiation of B-cells towards plasma cells.
The hypothesis that plasma cells had a pivotal role in refractory RA was further supported when we compared the efficacy and safety of two strategies inducing longstanding B-cell depletion in refractory RA patients in ***chapter 6***. We demonstrated that over a 48 weeks follow-up period both 'fixed' versus 'on-demand' retreatment with rituximab resulted in comparable efficacy as measured by ACR response, EULAR response, change in DAS28 and HAQ scores and radiographic progression. However, our study indicated that a 2nd course of rituximab resulted in significantly better clinical responses in moderate and non-responders when given as a fixed retreatment than as on-demand retreatment. However, in the fixed retreatment arm, 15% of patients with a persisting good response were retreated with minimal additional effect. Also, with respect to the safety profile, both treatment strategies were comparable. Altogether these data suggested that prolonged B-cell depletion was of additive value for patients with persistingly high disease activity after a first course of rituximab.

From a methodological point of view, treating patients with rituximab has led to the recognition that other pan B-cell markers besides CD20 need to be included in monitoring of RA patients treated with rituximab. In ***chapter 7***, we provided experimental evidence that isolated mononuclear cells from rituximab-treated patients can be falsely negative for the membrane-associated CD20 protein due to masking of the epitope by rituximab. However, CD19 expression on B-cells was not influenced by rituximab. Additionally, in ***chapter 8***, we demonstrated that not all pan-B-cell markers are equally sensitive and specific to identify B-cells. Most importantly, we showed that only 78.1% and 75.2% of CD22+ cells

were positive for CD19 and CD20, indicating that CD22 had a significantly lower specificity for B-cells as compared to the CD19 and CD20 markers.
Antibody production is crucial to the humoral immune system in RA. Not only because antibodies are the functional result of a humoral immune response, but also because the production of autoantibodies plays a pivotal role in the pathology of RA. In ***chapter 9*** we conducted a study to determine whether long-lived nondividing plasma cells reside within human tonsil and to determine factors that influence ongoing Ig secretion. In a tonsillar organ culture model, our study demonstrated that tonsillar tissue harbors a population of nonproliferating, long-lived plasma cells. These plasma cells survived in clusters surrounded by stroma, facilitating cell-cell contact and optimal use of autocrine and paracrine factors. Moreover, a significant proportion of IgA and IgG appeared to be secreted by such nonproliferating, long-lived plasma cells, some producing large amounts of immunoglobulin as shown by ELISPOT.

In contrast to rituximab, high dose chemotherapy followed by autologous hematopoietic stem cell transplantation (HDC+HSCT) results in complete lymphoablation and thereby non-specifically affects the humoral immunity. In ***chapter 10*** the health status of patients with previously refractory RA patients was analyzed during 5 years following HDC+HSCT. This study demonstrated a significant improvement of health status, notably in the first 9 months posttransplantation. Furthermore, the quality adjusted life years (QALYs) gained for RA patients treated with HDC+HSCT outweighed those for RA patients treated with conventional therapy when treatment related mortality was below 2.8%. Subsequently, in ***chapter 11***, we investigated whether humoral autoimmune responses in six refractory RA patients, whose dysregulated immune system was ablated by HDC+HSCT, underpinned their clinical responses. We demonstrated that reductions in ACPA-IgG levels were associated with clinically prolonged responses to HDC+HSCT. In addition, we showed that the susceptibility of ACPA-IgG levels to HDC+HSCT was associated with a high degree of synovial inflammation before treatment and presence of ACPA-IgG autoantibodies of low avidity.
At last, in ***chapter 12***, the arguments pro and contra a pathogenic role of anti-citrullinated protein antibodies (ACPAs) in RA are discussed. The hypothesis is presented that autoreactive plasma cells are critically involved in the pathogene-

sis of RA and, consequently, a better understanding of the biology of plasma cells in RA is advocated for future studies.

Conclusion

The aim of the present thesis was to investigate the role of the humoral immune system in RA patients. From a clinical perspective, this thesis addressed the issue of long-term treatment strategies for B-cell depletion and the results of a small pilot study suggested that both fixed as well as on-demand treatment with rituximab were equally effective in refractory RA patients. From a biological perspective, this thesis showed that a) B-cells of RA patients have an enhanced propensitiy to differentiate into plasma cells; b) the synovial load of CD79a+ plasma cells is associated with clinical disease activity before and after rituximab treatment and c) ACPA-IgM together with CD79a+ synovial infiltration could predict non-responsiveness to rituximab treatment. Altogether, these studies supported the hypothesis that autoreactive plasma cell in synovium are responsible for disease activity in refractory RA patients. Moreover, when investigating the effects of immunoablative therapy (HDC+HSCT), the clinical response was associated with the reduction, and in one case even eradication, of RA-specific autoantibody production, notably of low avidity ACPA. Therefore, it can be concluded that the deviations in humoral immunity in RA are concentrated towards autoantibody-producing plasma cells, suggestive that this subset plays a pivotal role in the pathological disturbances of the humoral immune system in RA.

Future Perspectives

Most autoimmune disorders are characterized by the presence of autoantibodies and abnormalities in B-cell function[1]. Autoantibodies may form immune complexes that engage other immune cells and can activate complement, triggering an inflammatory response. B-cells may also participate in immune responses by activating T-cells, secreting cytokines and influencing lymphoid structure[2]. Analysis of the mechanism(s) through which rituximab ameliorate(s) disease activity in RA forms a crucial step in deducting the role of the humoral immune system in RA.

Although autoantibodies are associated with disease severity and selected clinical phenotypes in RA[3], levels are not strong correlates of disease activity[4]. Rituximab treatment leads to variable decreases in both RF-IgM and ACPA-IgG, irrespective of improvements in clinical disease activity[5-7]. However, these autoantibodies are an insufficient reflection of the autoreactive plasma cell compartment as the different isotypes of ACPA and RF are neglected. Indeed, it has been previously described that circulating autoantibodies of all isotypes are present in RA and are continuously produced during the disease course[4]. Moreover, we found a strong association between ACPA-IgM and clinical non-response to rituximab[7]. These findings imply that the clinical benefits of B-cell depletion can not only find their immunological basis in the production of autoantibodies. Accordingly, tissue effects after rituximab treatment have provided additional information. Vos *et al.* demonstrated that rituximab resulted in rapid B-cell depletion in synovium and in later stages a reduction of other infiltrating cell types, including T-cells and macrophages, and lymphocyte aggregrations[8,9]. These data strongly suggested that the presence of B-cells orchestrated the characteristic synovial infiltration in RA. However, other anti-inflammatory mechanisms cannot be excluded. For instance, rituximab has also been demonstrated to influence survival factors for B-cells, thereby reducing survival niches such as inflamed tissue[10]. Altogether, these studies show that much work is still needed to understand the mechanism of action of rituximab in RA patients.

Autoreactive plasma cells are resistant to current immunosuppressive treatment regimens including biologicals. As discussed before, these plasma cells are crucial to the humoral immune system and the pathologic mechanisms involved in the formation and survival of autoreactive plasma cells in RA are largely unknown. Therefore, plasma cells may prove to have a more central role in RA pathogenesis than is currently appreciated. Therapeutically targeting autoreactive plasma cells may prove to be challenging as these cells are neither dividing nor proliferating nor are they identifiable by specific membrane-bound proteins[11]. Because plasma cells have a highly active protein metabolism to produce antibodies and consequently increased protein degradation, specific inhibitors of the proteasome are now being investigated as candidate therapeutics for RA[12,13]. Moreover, as a result of new insights into the molecular pathogenesis of multiple myeloma, a plasma cell malignancy, novel approaches to

target abnormally activated cascades in these plasma cells have recently been developed, including inhibition of membrane receptor tyrosine kinases, inhibition of histone deacetylases, inhibition of farnesyltransferases and targeting of molecular chaperones[14]. Additionally, therapeutic regimens for targeting plasma cells can be expanded even to the extent of allogeneic stem cell transplantation[15].

In conclusion, recently developing strategies targeting (autoreactive) plasma cells might prove to be of value in RA and other autoimmune disease in which plasma cell abnormalities have been observed[16-18]. Consequently, future studies will need to identify the immunobiology of autoreactive plasma cells which eventually may lead to new and effective treatments to achieve long-lasting control of disease activity in RA.

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