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Chapter 6

Summary and General Discussion
SUMMARY

The presence of anti-citrullinated protein antibodies (ACPA) is associated with clinical phenotype of rheumatoid arthritis (RA), especially with an enhanced rate of joint destruction. Several characteristics of ACPA, such as epitope spreading (1;2), isotype class switching (3-5) and Fc-glycosylation (6;7), are settled before disease onset and the presence of certain ACPA features might influence the clinical course of RA (8;9). In this thesis several characteristics of ACPA during RA development were explored.

1. Avidity maturation of ACPA responses in the pre-disease stage to established rheumatoid arthritis and its association with clinical phenotype.

Several observations implicate ACPA in disease pathogenesis. The presence of ACPA predicts the emergence and outcome of Rheumatoid Arthritis (RA) (10-12). Moreover, in vivo, ACPA can induce and aggravate arthritis in mice (13-15) and in vitro, it can activate human immune effector mechanisms, such as triggering of cellular Fc receptors (16;17) and activation of the complement system (18).

In a chronic course of RA, there is conceivably a continuous triggering of immune cells by citrullinated- (auto) antigens which is likely to impact on the differentiation of ACPA producing B cells (19). ACPA can use all immunoglobulin isotypes (20-22) and recognize protein antigens indicating the presence of helper activity to ACPA producing B by CD4+ T helper cells. The latter is also supported by the observation that the HLA-alleles predisposing to RA, predispose to ACPA positive RA and not to ACPA negative RA (23;24). During a B cell response, isotype switching and affinity maturation typically occurs in the germinal center. Following somatic hypermutation, different B cell clones will compete for antigen mostly on follicular dendritic cells (FDC). Conventional B cells expressing surface immunoglobulins with a higher avidity will acquire the signals necessary for survival and proliferation. As a result, the total avidity of the immune response increases because low avidity B cells will not be stimulated and will eventually disappear from the population. However, our data (in chapter 2) indicate that the ACPA response is different from antibody responses against recall protein antigens. The ACPA response can be of high titers and can use all isotypes yet is of low avidity, contrasting antibody responses against recall antigens that are of high avidity. The longitudinal follow up of ACPA avidity in patients with established RA did not reveal further avidity maturation in the progression of disease.

However, we noticed that although most RA patients were characterized by low ACPA avidity, also RA patients with a relatively higher ACPA avidity were present. We therefore, hypothesized that ACPA avidity maturation might occur before
disease onset and stabilizes thereafter. In chapter 3, we compared the ACPA avidity (cross-sectional study) of ACPA positive healthy (pre-disease) individuals, arthralgia patients, undifferentiated arthritis (UA) patients and established RA patients. We also performed longitudinal studies using samples that were collected before the onset of arthritis. Our data reveal a limited avidity maturation occurring before disease onset. Therefore these data provide further input to the growing body of evidence showing that the ACPA response matures before, but not after symptom onset.

Furthermore, we hypothesized that the degree of ACPA avidity may determine the clinical phenotype of RA. In chapter 4, we analyzed whether differences in ACPA avidity are associated with clinical outcome as determined by the rate of radiographic joint damage. In addition, the effector functions of low and high avidity ACPA were determined by analyzing immune-complex formation, complement activation and immune cells activation. Our study revealed that the presence of low avidity ACPA and not higher avidity ACPA is associated with a higher rate of joint destruction. These observations correlated with the finding that consecutive binding of low avidity ACPA to citrullinated epitopes was only weakly inhibited by either soluble- or solid-phase citrullinated antigens, in contrast to high avidity ACPA. Moreover, this effect correlated with a higher ability of low avidity ACPA to activate the complement system. Therefore, we believe that low avidity ACPA, as compared to high avidity ACPA, have an enhanced capacity to penetrate into tissues and recruit effector mechanisms locally providing a possible explanation for the association with an increased rate of joint destruction.

2. ACPA IgM response in early rheumatoid arthritis

In addition to ACPA avidity, the continuous presence of ACPA IgM (25) also differs from conventional humoral responses. The ACPA response likely represents a T-cell dependent B-cell response given the protein nature of the antigen recognized and the strong association with the HLA-shared epitope (SE) alleles. Therefore, the evolution of such a response is typically characterized by a first wave of IgM antibodies following first antigen contact, quickly followed by the presence of IgG. After repeated antigen exposure, the IgG responses are further boosted while the IgM peak declines because the half-life of circulating IgM is short. In addition, IgM-producing memory B cells against T-cell dependent antigens have not been described, in contrast to T-cell independent B-cell responses against, for example, repetitive sugar residues on bacteria (26;27). Therefore, it is most conceivable that the presence of ACPA IgM suggest activation of recently recruited B-cells recognizing citrullinated antigens.
We hypothesized that there might be certain antigens which continuously drive ACPA response in RA. As such, in chapter 5, we determined whether there is a difference in the fine specificity of IgG and IgM ACPA. Our data indicate that the immune response against several defined citrullinated antigens varies as some are only recognized by IgG ACPA and others also by IgM ACPA. Essentially, ACPA IgM displays a more restricted antigen recognition profile as compared to ACPA IgG. The recognition of some but not other citrullinated antigens by ACPA IgM suggests that not all citrullinated antigens are able to activate new B-cells despite concurrent recognition by ACPA IgG.

Conclusions
The ACPA response is different from conventional antibody responses. The ACPA response is generally of a much lower avidity than recall responses. Avidity maturation is limited and occurs before disease onset. The presence of low avidity ACPA is associated with higher rate of joint destruction. In vitro, the binding of low avidity ACPA to citrullinated epitopes was weakly inhibited by citrullinated antigens and low avidity ACPA have a higher ability to activate the complement system. In addition, there are certain antigens which continuously drive ACPA (IgM) response in RA.

GENERAL DISCUSSION AND FUTURE PERSPECTIVES

The reason why ACPA are of low avidity is not known, but it is conceivably related to the fact that ACPA recognize autoantigens. In contrast to vaccinations, where a small and limiting quantity of antigen is provided to induce an immune response, the autoimmune reaction is characterized by a (likely) excess of autoantigen. Moreover, unlike isotype switching, avidity maturation not only critically depends on the presence of antigen, but most likely also on proper amounts of antigen presenting in the germinal centers (28). If there is excess antigen, such as could be the case for autoantigens, there may be no proper selection for higher avidity as no competition for antigen will occur. This will result in an antibody response that does undergo isotype switching but does not display avidity maturation.

Most RA patients were characterized by low ACPA avidity. However, RA patients with a relatively higher ACPA avidity were also present. In addition, the clinical phenotype of ACPA positive RA is heterogeneous and low ACPA avidity is associated with a higher rate of joint destruction. Therefore, there might be a difference in the biological ‘potential’ of low- versus high ACPA avidity. Low
avidity antibodies require divalent binding to form immune complexes, which can only occur if antigen density is above a certain threshold. In contrast, high avidity antibodies can bind effectively with a single antigen-Fab interaction, irrespective to the antigen density (29). In rheumatoid arthritis, most of ACPA circulate in blood (30) or synovial fluid (31), while citrullinated antigens are enriched in synovial tissues (32). We hypothesize that relatively high avidity ACPA might be partially trapped in circulating IC before binding to target antigens in synovial tissues. In contrast, low avidity ACPA can easier detach from antigen, allowing subsequent binding to abundant antigens present in synovial tissue (33). In vitro, low avidity ACPA to citrullinated epitopes was only weakly inhibited by citrullinated antigens, in contrast to high avidity ACPA. This observation might translate to an higher penetrating capacity of low avidity antibodies, as also be shown for tumor antigens that are targeted monoclonal antibodies (34;35). Moreover, low avidity ACPA have a better ability to activate the complement system. When the Fc portions of two ACPA are close enough to bind C1q then complement is activated resulting in the covalent binding of complement fragments like C3b (36). Our data presented in Chapter 4 make it conceivable that low avidity ACPA in contrast to high avidity ACPA can bind, detach and rebind antigen (present at a different location), leading to more C3b deposition. These data would provide an explanation for the observation that RA patients harboring low avidity ACPA display a higher rate of radiological progression. Together, low avidity is one of the properties of ACPA possibly contributing to pathogenicity.

In addition, the ACPA response against one citrullinated antigen might differ from ACPA-responses reactive to other antigens. Some responses are dominated by IgG, whereas for other ACPA antigens both IgM and IgG responses are found. The presence of ACPA IgM was also a predictor of clinical outcome after anti-CD20 monoclonal antibodies (Rituximab) (37) and anti-TNF monoclonal antibody (Infliximab) (38) treatment. Elucidation of the mechanisms behind this observation could also be of relevance for the identification of those citrullinated antigens that drive ACPA-responses as well as provide clues on how the continuous recruitments of new B-cells could be halted.

In experimental models, Rituximab depletes B cells producing autoantibodies, while sparing the ‘conventional’ plasma cells producing the protective antibodies (39). Intrinsic differences between RA specific autoantibody system and the protective antibody responses against pathogens could be of relevance for designing novel B cell targeted therapies for RA.
REFERENCE LIST


(20) van der Helm-van Mil AH, Verpoort KN, Breedveld FC, Huizinga TW, Toes RE, de Vries RR. The HLA-DRB1 shared epitope alleles are primarily a risk factor for anti-cyclic citrullinated peptide antibodies and are not an independent risk factor for development of rheumatoid arthritis. Arthritis Rheum 2006; 54(4):1117-21.


(23) van der Helm-van Mil AH, Verpoort KN, Breedveld FC, Huizinga TW, Toes RE, de Vries RR. The HLA-DRB1 shared epitope alleles are primarily a risk factor for anti-cyclic citrullinated peptide antibodies and are not an independent risk factor for development of rheumatoid arthritis. Arthritis Rheum 2006; 54(4):1117-21.


