Tolerance and immune regulation in rheumatoid arthritis

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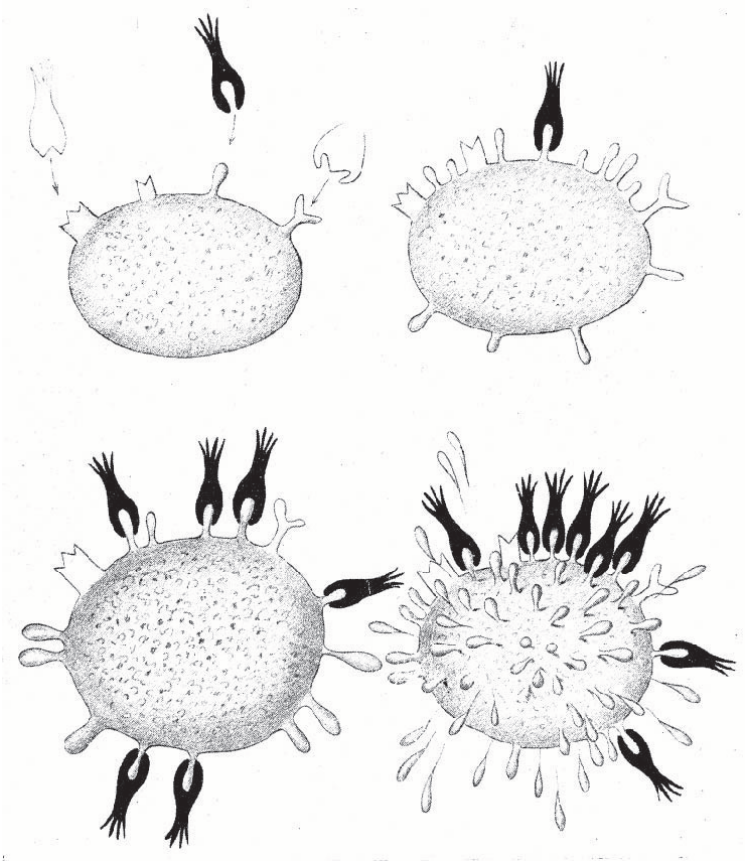
PART I

ROLE OF PROTEIN MODIFICATIONS ON AUTOIMMUNITY



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Chapter 2

The role of anti-citrullinated protein antibodies in the early stages of rheumatoid arthritis

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Introduction

The identification of anti-citrullinated protein antibodies (ACPA) has had a major impact on the understanding of rheumatoid arthritis (RA). In the late 1990's it was described for the first time that RA patients produce autoantibodies which target peptides and proteins containing citrulline, a modified form of the amino acid arginine (1) (2). Citrullination is a posttranslational modification of protein-bound arginine into citrulline residues which is mediated by peptidyl arginine deiminase (PAD) enzymes and is essential for the generation of antigens recognized by ACPA (3). Although the physiological role of citrullination is not precisely known, it is clear that this protein modification can occur during a variety of biological processes, including inflammation. Following the identification of citrullinated proteins, several diagnostic tests were developed based on cyclic citrullinated peptides (CCP) as a test substrate for detecting ACPA. Using the CCP-assay, a highly reliable diagnostic tool became available for routine testing of antibodies directed against citrullinated epitopes in early RA patients.

The presence of ACPA in the sera of patients represents an important early biomarker and has been added to the 2010 American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) classification criteria for RA (4). The selected parameters in the 2010 classification criteria were designed to include early markers of disease rather than established clinical features as was the case in the 1987 ACR guidelines. The ACPA serology enables the clinician to identify recent-onset RA patients earlier, which is crucial for achieving timely control of disease progression. Using the ACPA test it is possible to distinguish two subclasses RA patients: ACPA-positive and ACPA-negative. When comparing these two subclasses of RA, major differences have been observed regarding genetic- and environmental risk factors, progression, remission, and response to treatment. In this review, we will provide an update on the latest findings concerning the ACPA maturation profile, the association between RA and the HLA DR-locus, and the hypotheses about disease pathogenesis that contribute to a greater understanding of the role of ACPA in early RA.

Auto-immunity related to RA is present long before onset of clinical symptoms

Autoantibodies are an important hallmark of RA and several classes of autoantibodies have been described that precede the development of RA, including ACPA, rheumatoid factor (RF) and the recently identified anti-carbamylated protein antibodies (5-8). Especially ACPA are of particular interest as these autoantibodies are highly specific for RA and can be found in about 50% of early RA patients. The fact that ACPA are quite rare in healthy individuals, suggests that auto-antibody positive healthy individuals are at an increased risk of developing RA (9). These findings suggest that ACPA and/or the underlying B-/T-cell responses play a prominent role in disease pathogenesis. Shortly before clinical onset of

disease, there appears to be maturation of the ACPA response which is characterized by an increase in ACPA titre, isotype switching, an increased antigen-recognition profile, and a change in Fc glycosylation pattern (10-15). Different observations strongly suggest that the development of ACPA-positive RA is based on a two-hit model. Environmental triggers and epigenetic stochastic events are thought to play a role in the initial break of tolerance leading to the formation of ACPA. A 'second hit', such as an infection or other factors, triggers the expansion of the ACPA response, which occurs relatively short before disease manifestation (16). Epidemiological studies have indicated that the HLA molecules do not play a considerable role during the first hit, but mainly contribute to the second hit that enables the expansion of the ACPA response.

ACPA can recognize a variety of citrullinated antigens, including type II collagen, fibrinogen, vimentin and many other citrullinated proteins. An increase or shift of the antigen recognition profile, epitope spreading, can be a sign of maturation of the antibody response and disease progression. Epitope spreading is a hallmark of maturation of the ACPA response and is predictive for disease progression to early RA. After disease onset, the increased citrullinated epitope-recognition profile stabilizes and does not change anymore (10). A recent 2-year follow up study enrolling 316 early RA patients in a Swedish pharmacotherapy trial suggested that disappearance of particular ACPA reactivities may be associated with a good treatment response in early RA (17). These results differ from previous reports in which the ACPA fine specificity did not seem to correlate with disease activity, progression, or response to therapy (18-20). In the case of the response against recall antigens, antibodies undergo class switching, somatic hypermutation and affinity maturation to improve the immune reaction against the antigen.

The variable region of ACPA has undergone extensive somatic hypermutation, indicative of a T-cell-dependent B-cell response (21). The avidity maturation of ACPA however, appears to be different from recall antigens. As compared with antibodies against recall antigens, ACPA display a considerably lower avidity and the ACPA response shows only limited avidity maturation over time (22, 23). The presence of these low-avidity ACPA in RA patients is associated with a higher rate of joint destruction. ACPA can activate the complement system and can therefore play a role in the complement-mediated recruitment of inflammatory cells (24), which suggests that ACPA could be directly involved in the disease process. Moreover, ACPA-immune complexes combined with IgM or IgA RF can directly trigger Fcγ receptors on macrophages and mast cells leading to the production of proinflammatory cytokines which contribute to RA synovitis (25, 26).

Maturation of antibody responses leads to a shift in isotype which enables the activation of other immune effector mechanisms. ACPA can use multiple isotypes, and these ACPA isotypes are already present before onset of RA (27). In addition, the number of different ACPA isotypes is predictive for the development of radiological damage (28). Similar to the epitope-recognition profile, the ACPA isotype profile appears not to expand anymore during

disease progression, indicating that maturation of the ACPA response takes place before onset of arthritis. As mentioned above, the fragment crystallisable (Fc) region of an antibody interacts with Fc receptors of immune effector cells and the complement system, and thus determines which immune effector mechanisms can be recruited by the antibody. The glycosylation of the IgG-Fc region of ACPA has been reported to be different from non-ACPA IgG. The Fc region of ACPA-IgG_1 contains reduced numbers of sialic acid and galactose residues (29), a feature which is generally considered to render IgG antibodies proinflammatory (30). The changes in ACPA Fc glycosylation pattern become more prominent around 3 months before onset of RA (15). Differences in glycosylation pattern between Ig isotypes might influence their affinity for Fc receptors. A recent study showed that ACPA-IgG_1 has a different Fc glycan profile compared to non-CCP2 reactive IgG_1 (30), a particularity which can influence the affinity of ACPA IgG to Fc receptors and complement and may modulate ACPA effector- and immune-regulatory functions (31). In conclusion, all these different autoantibody characteristics evolve and mature before disease onset, and once patients present with arthritis, the ACPA response is generally increased in titre, uses more isotypes, displays a different glycosylation pattern, and are cross-reactive towards different citrullinated proteins (Figure 1).

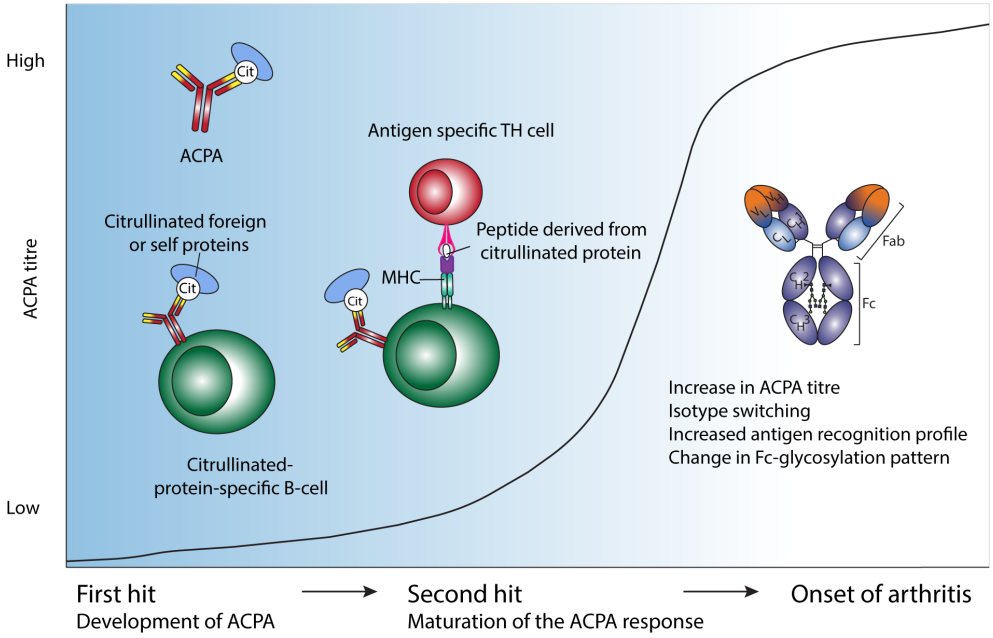

Figure 1. Maturation of the ACPA-response. Antibodies reactive towards citrullinated proteins are already present in the preclinical phase of RA. Environmental triggers and epigenetic stochastic events are thought to play a role in the 'first hit', leading to the formation of ACPA. A 'second hit', such as an infection, triggers further expansion and maturation of the ACPA response. Once the disease manifests itself, the ACPA response is generally increased in titre, uses more isotypes, has a different glycosylation pattern, and an increased antigen recognition profile towards various citrullinated proteins. Abbreviations: MHC, major histocompatibility complex; T_H, T-helper cell; Fab, fragment antigen-binding; Fc, fragment crystallisable.

HLA class II associations in rheumatoid arthritis

The most important genetic risk factor for ACPA positive RA is the HLA class II region. RA, like many other autoimmune diseases, is characterized by a strong association with variants in the human leucocyte antigen (HLA) class II region. These HLA-associations differ in ACPA-positive and ACPA-negative disease (32-35), highlighting the complexity of pathogenic mechanisms underlying HLA associations in RA. The HLA class II region encodes for HLA-DR, HLA-DQ and HLA-DP proteins and is involved in antigen presentation to HLA-class II restricted $CD4^+$ T-helper cells. In 1976, analysis of mixed lymphocyte cultures from RA patients revealed that these individuals had certain HLA-DR4 molecules in common (36). The HLA haplotypes that encode for the HLA-DR4 molecules were found to be characterized by the so-called 'HLA-shared epitope (SE)', a common amino acid sequence in the HLA-DRB1

chain (37). Recent genome wide analysis revealed specific amino acids at positions 11, 13, 71 and 74 of the HLA-DRB1 chain as well was single-amino-acid polymorphisms at position nine of HLA-B and HLA-DPB1 are associated with the greatest risk for RA (38). Amino acid positions 11 and 13 of HLA-DRB1 are among the most polymorphic and are highly relevant for the shaping of peptide binding pockets located in peptide-binding groove of the HLA molecule. It is therefore not surprisingly that the statistically significant amino acid positions are those involved in peptide presentation.

The HLA SE alleles, are now known to be specifically associated with ACPA-positive RA (38) (32). Conversely, HLA-DRB1*13 alleles haplotypes have been found to protect against the development of ACPA-positive RA (33, 34). A possible explanation for the association of the 'HLA-shared epitope' with ACPA-positive RA might be that peptide presentation by the 'HLA-shared epitope' HLA molecules can facilitate the activation of CD4+ T-cells which provide help to ACPA-producing B-cells. In ACPA positive RA, ACPA are cross-reactive and bind to a wide variety of citrullinated self-proteins which indicates to a loss of B-cell tolerance. However, it is unclear to what extent T-cell tolerance is lost. Identification of citrullinated epitopes recognized by autoreactive T-cells in patients with RA has proven difficult. Analysis using peptide-HLA tetramers and *in vitro* T-cell responses to candidate epitopes revealed T-cell recognition of several citrullinated epitopes in humans (39-42).

The HLA-DRB1*13 alleles which are protective for RA, carry a five amino-acid sequence called: DERAA. The DERAA sequence is also expressed by many microbes and in a self-protein vinculin. Citrullinated vinculin is expressed in the inflamed synovial membrane and was recently identified as a novel autoantigen for ACPA antibodies (43). It is proposed that molecular mimicry of self-proteins with pathogenic microbial proteins might lead to a break of T-cell tolerance. Indeed, it was recently shown, that T-cells present in HLA-DRB1*13-negative donors were able to specifically recognize a DERAA-containing vinculin epitope that cross-react with DERAA sequences derived from pathogens (44). However, many T-cell responses are absent in HLA-DR13+ donors, indicating the induction of DERAA-specific T-cell tolerance in these donors. Together, these studies suggests that the HLA class II locus can directly influence the maturation of the ACPA response via antigen-specific T-cells, providing help to ACPA-producing B-cells and enabling the maturation of the citrullinated protein-specific autoantibody response.

Pathogenic role of the immune response against citrullinated proteins

Besides the diagnostic application of ACPA as a biomarker, several clinical observations suggest that ACPA could play a direct role in disease pathology. First, ACPA may be found early in the course of disease, up to 7 years before RA manifests (5, 6). Second, various follow-up studies revealed that ACPA-positive patients with recent-onset RA develop more bone erosions compared to ACPA-negative RA patients (45-48). Third, bone loss and reduced bone mineral density can be found in healthy ACPA-positive individuals, even before clinical onset of arthritis (49). Selective B-cell depletion using rituximab has been found to be effective in the treatment of RA (50-52), providing evidence for the involvement of B-cells in the pathogenesis of RA. ACPA-producing B-cells are found to be enriched in synovial fluid (21, 53), which suggests that ACPA can be produced locally and directly contribute to synovial inflammation. Moreover, the numbers of ACPA-producing B-cells in the blood of RA patients correlate with ACPA serum levels (54).

Functional studies showed that immune complexes formed with ACPA mediate effector functions via Fc-γ receptors (55), and can induce complement activation (24) and enhanced neutrophil extracellular trap formation (56). In addition, there are reports that purified ACPA can induce osteoclastogenesis and bone resorption in mice (57), suggesting a direct link between ACPA and more severe joint destruction. So far, only two experimental studies succeeded in showing *in vivo* that ACPA may facilitate the transition from autoimmunity to inflammation. Transfer of antibodies specific to citrullinated fibrinogen (58) and transfer of antibodies targeting citrullinated-collagen (59) to mice with mild experimental arthritis led to disease exacerbation. It is interesting that no other positive data have been reported allowing that these two positive papers are similar as the many non-replicated preclinical papers in other fields such as oncology (60).

RA patients receiving Abatacept show reduced levels of ACPA and RF in response to treatment (61). Moreover, a Swedish pharmacotherapy trial reported a decline of all ACPA levels independent of the clinical response on disease activity during the first three months of methotrexate treatment (17). However, effective treatment of established arthritis does not necessarily lead to reduced ACPA levels or a change in ACPA composition. For example, a Canadian cohort in early arthritis patients found that anti-CCP antibody fluctuations did not relate to clinical scores such as disease activity scores and the presence of erosions (62). These findings suggest that autoantibody producing B-cells rather than the autoantibodies produced may be responsible for disease pathogenesis. Activated B-cells secrete pro-inflammatory cytokines, such as IL-6 and TNF, and ACPA-producing B-cells are found to be increased in the inflamed synovial membrane (16).

Besides ACPA, other autoantibodies and/or auto-antibody-producing B-cells may also be involved in RA pathogenesis. Similar to ACPA, both rheumatoid factor and anti-CarP antibodies are associated with disease severity and persistence (8, 63, 64). Anti-CarP

autoantibodies recognize carbamylated proteins containing a homocitrulline, a post-translational modification of lysine driven by cyanate, and can be found in patients with ACPA-negative RA. More recently, antibodies targeting another post-translational modification, malondialdehyde/acetaldehyde (MAA) adducts, are found to be increased in RA patients and this antibody response is associated with the presence of ACPA (65). Similar to carbamylation, MAA adducts are capable of the modification of a lysine. These findings raise the question why RA patients produce auto-antibodies towards post-translational modified proteins and whether these autoantibodies are implicated in disease pathogenesis. Further research is needed to confirm the current observations of anti-CarP antibodies in ACPA-negative and positive RA patients, and to determine whether these biomarkers can provide additional value next to the CCP2 test.

Conclusion

ACPA have proven to be a very useful biomarker for diagnosing RA and for predicting a severe disease course. Future investigations on the role of ACPA, other autoantibodies, and ACPA-producing B-cells in RA may provide further insight in and understanding of the underlying disease pathogenesis. Follow-up studies of RA patients may provide useful information on the fluctuation of ACPA levels and changes in ACPA composition during disease progression and treatment. Together, these observations may allow for new approaches to treat RA at an early, preclinical, stage of disease, and thus enable prevention of the transition of autoimmunity to inflammation and autoimmune disease.

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