How is autoimmunity against citrullinated proteins regulated?
Cantaert, T.

Citation for published version (APA):
English summary

Anti-cirullinated protein antibodies (ACPA) are autoantibodies reacting against citrullinated protein epitopes. These are generated during posttranslational modification of a protein by a peptidyl arginine deiminase (PAD) enzyme, which is a natural process in healthy individuals. The precise function of these modifications is not entirely understood but, as it involves changes in protein charge, it can modify the protein’s structure and interactions with other molecules. In rheumatoid arthritis (RA), about 60-70% of the patients develop antibodies specifically directed against citrullinated epitopes. This break in tolerance against citrullinated proteins is highly specific for RA as the antibodies have a specificity of 95-98%. In this thesis, we investigated the mechanisms contributing to the development of these autoantibodies in humans using different strategic approaches. Firstly, we took example of other autoimmune diseases with more readily defined autoantigens. Secondly, we used the existing knowledge from different animal models and assessed if similar mechanisms were also relevant for B cell tolerance against citrullinated proteins in human RA. Furthermore, we focused our attention to the inflamed synovial membrane as ACPA can be produced locally in the joint.

In the first two chapters, we investigated intrinsic defects in B cell tolerance mechanisms. We previously observed that treatment with tumor necrosis factor (TNF) blockers induced anti-nuclear antibodies in spondyloarthritis (SpA) patients, an immune mediated inflammatory disease without any known autoantibodies. In the first chapter, we pinpointed the factors contributing to this break in B cell tolerance. Not type I interferon (IFN), but very high levels of the antigen, nucleosomes, correlated with the induction of the antibodies. This seemed to be initiated by a defect in clearance of the nucleosomes rather than a massive elevation of the availability of the antigen. In the second chapter, we further investigated the influence of type I IFN on ACPA and rheumatoid factor (RF) levels. We found that the type I IFN signature had no significant influence on autoantibody levels. Interestingly, also healthy individuals with ACPA who are at risk of developing RA did not display an altered type I IFN signature. These data indicate that type I IFN is not a major determinant of the breach of B cell tolerance towards citrullinated proteins in RA.

In chapters three to six, we studied the presence of citrullinated epitopes and the citrullinating PAD enzymes in RA. In the third chapter, we described the presence of intracellular citrullinated proteins in the synovial tissue of RA patients. Importantly, these epitopes were not detected in the inflamed synovial tissue of other inflammatory arthritides. They co-localized with PAD2 immunoreactivity and were associated
with higher local and systemic ACPA levels. With these data we emphasized the importance of not merely citrullinated proteins, but specific citrullinated epitopes in the generation of the ACPA response. In the fourth chapter, we assessed the biological relevance of reported haplotypes of the PAD4 enzyme, which influence the mRNA stability and thus possibly the amount of enzyme, for the development of the ACPA response. In line with our findings in chapter 1, it was hypothesised that augmented presence of citrullinated proteins induced by PAD4 polymorphisms could break a B cell tolerance threshold. However, we did not observe any differences in ACPA levels or presence of synovial intracellular citrullinated proteins in patients with different PADI4 haplotypes. In chapter five, we observed the presence of citrullinated proteins in target organs other than synovium: RA-associated interstitial pneumonia and rheumatoid nodules. We also observed the presence of citrullinated proteins in inflamed lung biopsies of non-RA patients, confirming previous reports showing augmented citrullination in inflammation but at the same time questioning the relevance of these proteins in the initiation of the ACPA response. In our review in chapter six, we emphasized that well-defined citrullinated epitopes rather than citrullinated proteins as such are relevant for the break in B cell tolerance.

In the last two chapters, we investigated if specific immunological mechanisms required for optimal and coordinated immune activation are present in the RA synovial tissue microenvironment. Ectopic lymphoid neogenesis and germinal center like reactions have been proposed to promote humoral immunity and, eventually, autoimmunity. We explored the relevance of these structures in chapter seven. Our data demonstrated that synovial lymphoid neogenesis is a reversible and dynamic process which leads to true germinal center like reactions only in a minority of inflamed synovial tissues. Moreover, synovial lymphoid neogenesis was equally observed in seropositive RA, seronegative RA, and SpA, and was not associated with elevated levels of ACPA in the synovial fluid of the same joint in RA. Finally, we did not detect antigen-driven clonally expanded or affinity maturated B cells in tissues with these structures. In the last chapter, we provided molecular evidence for the involvement of synovial T cells in the pathogenesis of ACPA positive RA. Although clinical disease features and synovial histopathology were similar in ACPA positive and negative RA, clonally related synovial T cells were specifically increased in the former subset. These clonal expansions are specific for the synovial compartment as they were not found in peripheral blood.

In conclusion, the answer to the question asked in this thesis “How is autoimmunity against citrullinated proteins regulated” is complex and not yet fully clarified. We show here that multiple factors are involved in the development of the ACPA response, including intrinsic defects in B cell tolerance, specific features of the
citrullinated antigens, and immunological factors such as the shared epitope and T cell involvement. Other factors such as type I IFN, PADI4 polymorphisms, and ectopic lymphoid neogenesis do not directly influence the break in B cell tolerance against citrullinated epitopes.