Unravelling the anti-carbamylated protein antibody response in rheumatoid arthritis

Verheul, M.K.

Part III

Addendum

Summary

Chapter 1 introduces rheumatoid arthritis (RA) as a complex, chronic autoimmune disease. It is unknown how this disease develops but both environmental (smoking) and genetic (HLA) risk factors have been discovered to play a role. Many treatment options have been developed that make RA more bearable, but no cure is available at the moment. However, very early treatment of the disease provides good results and may help to cure RA. This would however require early diagnosis or even prediction of disease development. Currently, rheumatoid arthritis is diagnosed based on joint inflammation and serum biomarkers such as autoantibodies. The autoantibodies currently most often used for diagnosis are rheumatoid factor (RF) and anti-citrullinated protein antibodies (ACPA).
Chapter 2 describes the multitude of autoantibodies present in RA and psoriatic arthritis patients. Most of the antibodies described are only present in one of the two diseases and could be used to distinguish between RA and psoriatic arthritis. With regards to RA, one of the most studied antibody group that is not yet in use in the clinic are antibodies that target the post-translational modification carbamylation (anti-CarP antibodies). Therefore, the first part of this thesis focuses on the clinical role of anti-CarP antibodies and how these antibodies compare to ACPA and RF which are currently more commonly used in the clinic.

Chapter 3 investigates whether some of the RA-specific autoantibodies (RF, ACPA and anti-CarP antibodies) are also present in an animal model of RA. Surprisingly, anti-CarP antibodies could be detected in a rhesus macaque model of collagen-induced arthritis, while ACPA and RF were undetectable. Also, these anti-CarP antibodies were often present before the onset of clinical symptoms. Importantly, some animals without clinical symptoms did develop anti-CarP antibodies and some animals that were ill showed no detectable anti-CarP antibody levels.
Chapter 4 on the other hand analyses the presence of anti-CarP antibodies in the human situation. Previous measurement of these autoantibodies were restricted to Caucasian populations. This chapter provides the first evidence that anti-CarP antibodies are also present in an Asian population of RA patients. The distribution of anti-CarP antibodies in this cohort of Japanese RA patients is rather similar as observed in the Caucasian population, indicating that the measurement of anti-CarP antibodies may be relevant in a worldwide setting.
Chapter 5 investigates the presence of anti-CarP antibodies and several ACPA fine-specificities in a large cohort of RA patients, in which serum samples before and after the diagnosis of RA was available. It is shown that also in the human situation, anti-CarP antibodies can be detected many years prior to diagnosis. Anti-Carp levels and positivity are higher in pre-symptomatic individuals when compared to controls. A further increase in levels and frequency of anti-CarP antibodies could be observed after diagnosis of RA. Furthermore, it was demonstrated that anti-CarP antibodies associate with disease development and joint damage (also in ACPA-negative individuals).

Besides an increased presence of anti-CarP antibodies in RA patients, these antibodies may also be present in other diseases.
Therefore chapter 6 describes the presence of anti-CarP antibodies in conditions with increased carbamylation, namely smoking, renal disease and chronic inflammation. When compared to healthy controls, a slightly increased prevalence of anti-CarP antibodies could be observed for renal disease, but the percentage of antibody-positive RA patients was much higher than for any of the other conditions. Since the presence of anti-CarP antibodies has been measured in a large number of cohorts, a meta-analysis based on these data was carried out in Chapter 7. The general aim of this study was to investigate whether anti-CarP antibodies could add to the diagnosis or prediction of RA, especially when taking into account that ACPA and RF are already in use in the clinic. For the diagnosis of RA, cohorts with RA patients, disease controls, healthy first-degree relatives and healthy controls were analysed. The results indicated that ACPA and RF may be sufficient to diagnose RA patients. To investigate the prediction of RA development in healthy individuals, we analysed three independent cohorts including the pre-symptomatic individuals, as in chapter 5. In this case, the measurement of ACPA, RF and anti-CarP antibodies provides an increase in the odds of developing RA, indicating that the combination of 3 autoantibodies may assist in prediction and early diagnosis of RA.

Although much information is available on the relationship between the presence of anti-CarP antibodies in relation to clinical presentation of RA, less is known on the antigens and the development of anti-CarP antibodies. These are the main subjects of the second part of this thesis.
Chapter 8 focuses on the detection of carbamylated and citrullinated proteins. Since the amino acids homocitrulline and citrulline, resulting from these reactions show similarities, it may be difficult to distinguish between the two. For example, most commercially available antibodies marketed to recognize citrulline also recognize homocitrulline in both ELISA and western blot. The tested homocitrulline-targeted antibodies on the other hand preferentially recognize carbamylated proteins. Therefore, mass spectrometric analysis – if carried out carefully – seem essential for the identification of citrullination and carbamylation in complex protein mixtures.
In Chapter 9 further research into potential carbamylated antigens is carried out. The mixture of carbamylated fetal calf serum (Ca-FCS), currently used in ELISA for the detection of anti-CarP antibodies, is investigated in more detail. One of the proteins that is well-recognized by anti-CarP antibodies in the sera of RA patients is carbamylated alpha-1-antitrypsin (Ca-A1AT). When investigated in further detail, Ca-FCS and Ca-A1AT both perform similar when it comes to the identification of RA patients. Also, several Ca-A1AT peptides can be recognized as well, although this is less universal that Ca-A1AT protein recognition. The data from this chapter may be used for further optimization of diagnostic tests to measure anti-CarP antibodies.
Further investigation into antigens for anti-CarP antibodies in RA patients is carried out in chapter 10, where carbamylation is determined in joint tissue of RA patients and controls, using mass spectrometry.

Even though it was expected that carbamylation in RA joint tissue would be increased, the number of unique carbamylated peptides identified was similar between RA and controls. However, when comparing cartilage, synovium and synovial fluid, more peptides were identified in cartilage tissue. Importantly, the identified carbamylated proteins could be recognized by anti-CarP antibodies. Combined, these data indicate that anti-CarP antibodies can potentially contribute to disease progression by binding to carbamylated proteins present in the joint of RA patients.
Finally chapter 11 describes studies on further characteristics of anti-CarP antibodies and their antigens. It is demonstrated that antibodies in the sera of RA patients are able to recognize a large variety of carbamylated proteins. Furthermore, quite some cross-reactivity towards different carbamylated proteins is observed, although the actual cross-reactivity pattern is different for each patient. Interestingly, mice, when immunized with a carbamylated self-protein also show high cross-reactivity towards other carbamylated proteins. Moreover, immunization with a foreign carbamylated protein results in a break of tolerance towards carbamylated self-proteins, indicating that an immune reaction towards a foreign carbamylated protein may result in a general autoimmune response targeting both foreign- and self-proteins in caramylated form.

In the discussion, Chapter 12, several possibilities in which anti-CarP antibodies may contribute to RA and to acquiring further knowledge on this chronic disease are described. From a clinical perspective, future research should focus on the use of anti-CarP antibodies in the prediction of RA, using large prospective studies. Furthermore, many aspects with regards to the development, function and role of anti-CarP antibodies remain unclear. Unraveling these aspects may prove difficult but may also provide further insight into the development of RA and possible treatment options for RA patients.