Unravelling the anti-carbamylated protein antibody response in rheumatoid arthritis

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Chapter 12

Discussion

Rheumatoid arthritis (RA) is a disease that results from a complex interaction between genetic factors and the environment, resulting in chronic inflammation. There are many different immunological processes that may play a role in the development and continuation of RA. One of these immunological aspects is represented by autoantibodies[1] (Chapter 2). A subgroup of these antibodies, anti-carbamylated protein antibodies, were the main focus of this thesis, with the first aim focusing on the clinical relevance of anti-CarP antibodies.

The clinical role of autoantibodies in rheumatoid arthritis

To investigate the role of anti-CarP antibodies, their presence has been measured in a large variety of cohorts, providing information on their role in RA and other (related) diseases[2-11]. Many studies (including chapters 4 and 5) have confirmed that anti-CarP antibodies are present in RA patients and that their levels are increased when compared to control groups[9,12]. In chapter 5 further confirmation was provided for the presence of anti-CarP antibodies before disease onset and for the association between anti-CarP antibodies and joint damage. However, to determine the clinical value of anti-CarP antibodies, it is important to take into account the antibodies that are already in use in the clinic. Therefore a meta-analysis incorporating ACPA and RF as well was carried out in chapter 7 . Combining all data available indicated that using anti-CarP antibodies for the diagnosis of RA, in individuals already presenting clinical symptoms, may not add significant value since ACPA and RF combined perform as well on their own as with the addition of anti-CarP antibodies. Adding another measurement may therefore not be very cost-effective. However, when it comes to the prediction of RA, the addition of anti-CarP antibodies increases the odds at having / developing RA, with a very high specificity and at the cost of a lower sensitivity. This suggests that a in group of people positive for the combination of 3 autoantibodies contains a large proportion of potential future RA patients, which could be treated early to prevent disease development[13]. However, the studies investigated in the meta-analysis were all retrospective. In the future a large population-wide prospective study would be required to confirm the current observation that these 3 autoantibodies may be used to predict RA development. Besides these observations, it is also important to investigate whether anti-CarP antibodies may provide additional help to predict the treatment response in RA patients. Although a small study has been carried out into this direction, at this point no clear evidence is available to indicate that anti-CarP antibodies may contribute to these predictions[7].

Another aspect with regards to anti-CarP antibodies and associations that may warrant further investigations are genetics. One study, focusing on particular SNPs known to associate with RA, did not provide clear evidence for a correlation, at least not independently from ACPA[9]. Further information on the genetic components that relate to anti-CarP antibody positive disease may help to provide more insights into the mechanism and clinical consequences of anti-CarP antibodies.

Furthermore, it is important to note that many of the clinical associations that have been identified for anti-CarP antibodies are similar for ACPA, even though these autoantibodies are not the same subset and can be found independently in RA patients[12,14](Chapter 11). Besides these two more well-investigated autoantibodies, other autoantibodies have been identified in RA patients as well. Interestingly, attention is moving towards antibodies that target other post-translationally modified proteins[1]. Two of these are malondialdehyde modifications and acetylation[15-17]. Also for autoantibodies targeting these PTMs, there are several indications that they associate with joint damage and that they may behave in similar manner as ACPA and anti-CarP antibodies with regards to clinical associations. It should be noted that quite some overlap has been observed in RA patients in regards to autoantibody positivity towards PTMs. It is conceivable that more anti-PTM antibodies will be discovered in the future and it will become increasingly difficult to distinguish between the individual autoantibody subsets. Moreover, when combining all of these autoantibodies in RA patients, there remains a substantial group of patients that is negative for all autoantibodies. It therefore seems that – as has been argued by other as well[18,19] – RA may always exist of at least two distinct subsets: autoantibody-positive and autoantibody-negative. The clinical associations discussed above and the further speculations are therefore mainly relevant for the autoantibody-positive subset of RA patients.

The development of autoantibodies in RA

The fact that so many anti-PTM antibodies are able to develop in RA patients, raises the question how these autoantibodies arise. For ACPA, some theories have been formed based on genetic associations, for example with the HLA alleles[20]. However, these theories are difficult to prove, since most *in vivo* animal models lack ACPAs[21]. Anti-CarP antibodies on the other hand are common in animal models[21,22](Chapter 3) and are therefore quite suitable for further investigation into the development of autoantibodies in RA. Initial studies involving mouse models show that anti-CarP antibodies can, surprisingly be induces in a simple collagen-induced arthritis model, in which anti-CarP antibodies actually arise before disease onset[23]. Also, several other models of RA that involve the adaptive immune system show spontaneous development of anti-CarP antibodies[21]. These data seem to indicate that anti-CarP antibodies develop relatively easily when the adaptive immune system is activated.

In these systems, it is unclear which type of antigen is involved or how these arise. One possibility for the creation of carbamylated proteins is the combination of myeloperoxidase and thiocyanate, resulting in carbamylation in inflammatory environments[24]. Possibly, proteins that are normally inaccessible may become carbamylated under such inflammatory conditions and result in the development of anti-CarP antibodies. However, during life, many inflammatory situations are present and most people do not seem to develop these autoantibodies, as evidenced by the absence of anti-CarP antibodies in most healthy control populations[12,25].

Also, chapter 6 describes that a large increase in carbamylation due to inflammation or due to increase in urea does not explain the large amount of anti-CarP antibody-positive individuals in the group of RA patients, indicating that increased carbamylation alone is not sufficient for the development of anti-CarP antibodies in the human situation.

An interesting aspect is that chapter 11 describes that once tolerance is broken towards carbamylated proteins, many other carbamylated proteins can be recognized as well. The inducing protein can be a self-protein or a foreign protein, but both can result in recognition of carbamylated self-proteins and may eventually result in autoimmunity. Anti-CarP antibodies in human sera also show high cross-reactivity towards multiple carbamylated proteins, although to a lesser extent when compared to immunized mice. However, a break of tolerance towards carbamylated proteins due to the in vivo carbamylation of foreign pathogenic proteins seems to be an interesting working hypothesis.

A final aspect to take into consideration with regards to the development of anti-CarP antibodies is the low affinity of these antibodies when compared to immunization responses (unpublished data, Myrthe van Delft). The low affinity may suggest that we are at this moment not looking at the "original" antigen that is recognized by these antibodies or that the process to mature B cells producing anti-CarP antibodies differs from the classical immune response. Whether this is the case and which of these hypothesis may be true remains an interesting, but difficult topic for future investigations.

The possible functions of anti-CarP antibodies

Although it is unknown how anti-CarP antibodies develop in human RA, we do know that they exist. Therefore, the function of anti-CarP antibodies is interesting to investigate, especially in light of the clinical associations. At first sight, there is no reason to assume that the function of anti-CarP antibodies differs from other autoantibodies. A large amount of antibody-dependent functions has been described in autoimmune diseases[26,27]. This includes examples were antibodies can activate receptors such observed in Graves' disease or occasions where antibodies act as an antagonist, blocking receptor signaling such as in myasthenia gravis. Although it would be interesting if anti-CarP antibodies would have such actions, no evidence is available to support this. It is possible that the main mode of action of these antibodies in autoimmunity is related to their respective antigens, as investigated in chapter 10. These data indicated that a large amount of different proteins can function as antigen for anti-CarP antibodies. Also, it seems that carbamylation is present in many different tissues and may even be present throughout the entire human body. However, the longevity of cartilage (and other matrix proteins) may provide more time and opportunity to accumulate such modifications, as our observations indicated more carbamylation in cartilage tissue. Therefore, such tissues are the most likely targets of these autoantibodies.

Upon binding a (highly) carbamylated tissue, several actions could be mediated by anti-CarP antibodies. One of the first such mechanisms is through the complement system, via C1q binding[28]. This can result in the attraction of other immune cells due to the creation of chemoattractants C3a and C5a during the activation of the complement cascade. Whether the amount of carbamylation in cartilage, combined with the low avidity of anti-CarP antibodies is sufficient for complement activation is unknown but would be important for future investigations. However, low avidity ACPA seem to be better at complement activation than high avidity ACPA[29], which could also be the case for anti-CarP antibodies. A second mechanism through which anti-CarP antibodies may have an effect is by binding of Fcy receptors on the surface of several cell types. If bound to activating receptors (all but FcyRIIb) this may result in local activation of immune cells, dependent on the type of Fc-receptor bound and on which cell this receptor is present[30]. Eventually, this could lead to a local inflammatory reaction, for example in the joint.

Another effect related to anti-CarP antibodies that should be considered is that these antibodies do not only function as fluid-phase antibodies, but also as a B cell receptor. Binding of a B cell receptor to a carbamylated antigen may also result in B cell activation and production of inflammatory cytokines such as TNF-α and IL-6, which may contribute to an inflammatory response as well[31].

Combined, it seems that there are many ways in which anti-CarP antibodies may exert their function. How much each of these pathways contributes to the development or prolongation of the immune response would be important to understand, especially with regards to possible targeted therapies to stop this particular process in RA patients.

Anti-CarP antibodies in the pathogenesis of RA

Although there are many functions by which anti-CarP antibodies could potentially contribute to the pathogenesis of RA, it is at this point unclear what their contribution is towards the development or chronicity of RA. A first conclusion that can be made is that anti-CarP antibodies are not essential for the development of RA in general, since there is a percentage of patients without anti-CarP antibodies or antibodies in general, as discussed in chapters 4, 5, 6 and 7. However, autoantibody-negative RA as such may have to be considered as different disease subset.
A second conclusion that can be made is that anti-CarP antibodies do not directly lead to RA as anti-CarP can also be found in small subsets of individuals suffering from other diseases or in the healthy population. This does not exclude that anti-CarP antibodies could play a role in the development of RA, through many different disease pathways. It is therefore interesting to investigate the potential role of anti-CarP antibodies in the autoantibody-positive subgroup of RA patients.

The fact that anti-CarP antibodies are present in animal models might make it easier to investigate their role in RA[21-23]. In chapter 11, mice have been immunized with different carbamylated proteins, upon which they start to produce high levels of anti-CarP antibodies (when compared to the more natural production of anti-CarP antibodies in CIA mice). These antibodies are able to recognize a large amount of carbamylated proteins, but the mice do not show any obvious signs of arthritis, indicating that high levels of anti-CarP antibodies alone are not sufficient for the induction of disease in this model. It should be noted though that the mice used for these experiment are relatively young, while carbamylation is thought to increase with ageing[32]. Older mice may therefore be more suitable for pathogenic experiments, with regards to anti-CarP antibodies. This situation may also be equal for humans, in which RA is developed at a later age as well[33].

Another model in which anti-CarP antibodies are present is shown in chapter 3, where anti-CarP antibodies are investigated in rhesus monkeys after collagen injections aimed to induce CIA. Only part of the animals developed disease and also only part of the animals developed anti-CarP antibodies during the observation period. However, these two groups did not match: some monkeys did develop anti-CarP antibodies but showed no signs of disease, or the other way around. This indicates again that anti-CarP antibodies do not necessarily induce disease.

The current data on animal models in general do not show an obvious effect of anti-CarP antibodies on the development of arthritic symptoms[21]. This may indicate that this is also the case in human RA, but translation of data found in animal models to the human situation should be carried out with caution. However, the presence of anti-CarP antibodies years before disease onset, already indicates that anti-CarP antibodies alone are not directly pathogenic in humans as well[34,35](chapter 5). However, given their possible functions, it seems probably that anti-CarP antibodies play a role in disease propagation and that they may contribute to the inflammatory cascade in the joint, once initiated by other mechanisms. Also, one could speculate that the disease process in general is quite slow and that it takes quite some time to develop a disease with clinical presentation after initiation of the disease process.

Conclusion and future perspective

At this point, we know that anti-CarP antibodies are present in RA patients in many different cohorts around the world. These autoantibodies may arise during an infection or other immune response, due to changes in carbamylation or due to the carbamylation of specific proteins. Once created, anti-CarP antibodies may function through complement activation, Fcy-Receptor binding or by functioning as a B cell receptor activating B-cells. Due to these functions anti-CarP antibodies may contribute to chronic inflammation in RA and increase disease severity.

From a clinical perspective, anti-CarP antibody measurements may not add to disease diagnosis once clinical symptoms occur, but may provide additive value as a prognostic factor in combination with ACPA and RF. Future clinical research should therefore focus on these three autoantibodies in large population-wide prospective studies to determine the value of anti-CarP antibodies, ACPA and RF in predicting the development of RA.

With regards to the development and role of anti-CarP antibodies, many aspects are yet unclear and unravelling these processes will be difficult. Potentially, the key to finding the solutions for these problems is found in (autoantibodies in RA that target) other post-translational modifications (PTMs). For example, not all PTMs result in autoantibody development in RA. Studying differences between these modifications may help understand why autoantibodies do develop towards some of these modifications. Also, more detailed studies into different autoantibody subsets based on clinical associations, subclasses, isotypes and sequences may provide more insight into the function of these autoantibodies, especially when focusing on the differences between the subsets.

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