Development of Rheumatoid Arthritis
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Summary and Discussion
INTRODUCTION

The aim of this thesis was to investigate whether and how arthritis development can be predicted in individuals at risk for developing rheumatoid arthritis (RA) and to gain more insight in the evolvement of the disease.

Early treatment of patients with RA leads to greater response to therapy than delayed treatment and even results in sustained benefit (the 'window of opportunity').\(^1\text{--}^6\) Therefore, early diagnosis is important and numerous studies report the characteristics of early arthritis patients that developed chronic and/or erosive RA. Thereby these studies provide guidance in making treatment decisions in this early stage of disease. Recently, an American College of Rheumatology (ACR)/European League Against Rheumatism (EULAR) collaborative initiative resulted in new set of criteria for the early classification of RA.\(^7\)

Despite this focus on recognizing RA patients in an early stage, there are still patients that already have erosions at the moment they present with early active RA or develop them shortly after diagnosis.\(^9\text{--}^11\) Inflammatory processes that cause damage thus seem to be active already before arthritis becomes clinically evident. Focusing at this pre-clinical stage and treating patients in this stage might help to prevent more damage or even the development of RA altogether.

ACPA AND OTHER ANTIBODIES IN THE DEVELOPMENT OF RA

To gain more insight in the evolvement of RA and the role of anti citrullinated protein antibodies (ACPA), the development of these antibodies prior to RA diagnosis and their characteristics were studied.

ACPA are thought to play a pathogenic role in RA and have been studied extensively in established disease. It was shown that in this phase of the disease ACPA already target a multitude of different citrullinated antigens.\(^12\text{--}^13\) Therefore, development of ACPA probably takes place at an earlier stage and indeed, these antibodies could be detected in healthy blood donors years before they were diagnosed with RA.\(^14\text{--}^15\)

Moreover, Van der Woude et.al. were the first to report that ACPA epitope spreading, in which over time more and more antigens are recognized,\(^16\) takes place before disease onset.\(^17\) However, because in this study only one or two pre-disease samples were available, how the ACPA response evolved could not be investigated. In chapter 2, the results are reported of a study of ACPA development in healthy blood bank donors who later developed RA and of whom multiple sequential sera were available prior to diagnosis. It was shown that the ACPA response starts in a
very restricted manner with reactivity towards only one or two autoantigens, which is not always the same antigen. During this preclinical phase epitope spreading occurs over the course of several years and the numbers and levels of ACPA slowly increase. A few years before diagnosis a sharp rise in ACPA titers was observed. This phenomenon suggests a second stage in disease development, which might be due to a variety of factors, but supports the theory that a ‘second hit’ is necessary to progress from a deregulated immune system to actual RA.

In chapter 3 it was shown that the broadness of the ACPA response is predictive for the development of arthritis in arthralgia patients. Patients that recognized 2 or more peptides had a higher chance of developing arthritis than patients that recognized none or a single peptide. These results may indicate that reactivity directed towards more than one epitope could be essential for the development of chronic disease. In a collagen-induced arthritis (CIA) mouse model, arthritogenicity was substantially higher when a cocktail of five monoclonal antibodies recognizing different epitopes of collagen was used, then when a cocktail with lower number of monoclonal antibodies was used, supporting this hypothesis.

In chapter 4 a method is presented to clone monoclonal ACPA from an RA patient. With this method, two monoclonal ACPA were derived which were shown to be specific for citrullinated fibrinogen, but were also cross reactive with other citrullinated peptides, derived from other proteins. These cross reactive properties could indicate that ACPA are able to cross link antigens to each other into large immune complexes. The higher the number of different ACPA, the larger the immune complexes can be and therefore more immunogenic. These findings also support the hypothesis that crossing a threshold in the number of ACPA may be necessary to develop RA. In addition, it was observed that the avidity of ACPA was not high, a phenomenon that was also described by Suwannalai et al. ACPA avidity is generally lower than the avidity of classical recall antibodies.

Although avidity is generally low, it does rise towards the point of RA diagnosis as is described in chapter 5. Maybe, an abundance of antigen formed in the inflammatory process causes the avidity maturation to cease in favor of epitope spreading. I.e. the ACPA response may not mature in the same way as a classical recall response to for instance tetanus toxoid, because the response does not become focused towards one or only a few epitopes, but instead broadens towards a multitude of epitopes. It is tempting to speculate about a mechanism that causes this ‘maturation drift’, for instance altered regulation of somatic hypermutation.
However, it is not known whether such mechanisms play a role in the pathogenesis of RA.

ACPA are not the only antibodies found in RA. Of course many patients also have rheumatoid factor (RF). In the presence of ACPA, RF enhances the chance of developing arthritis in arthralgia patients and therefore RF may also play a role in the pathology of RA. RF can certainly contribute to the formation of large immune complexes which may be more capable of inducing inflammation than smaller complexes.

Other antibodies can be found as well. In chapter 6, the presence of a new antibody family, anti carbamylated protein antibodies (anti–CarP), is described. These antibodies are directed towards carbamylated proteins instead of citrullinated proteins. Carbamylation is a process in which lysines are converted under the influence of cyanate into homocitrullines, which highly resembles citrulline. Cyanate can be formed in low concentrations from urea under physiological conditions or it can originate from the environment, for instance from car fumes. In inflammatory conditions it can be formed from thiocyanate catalyzed by myeloperoxidase released by for instance activated neutrophils. Therefore, carbamylation just as citrullination, will be more abundant in inflammatory conditions. In chapter 6, it was found that Anti–CarP antibodies can be detected prior to the diagnosis of arthritis and that they predict the development of arthritis independent of ACPA and RF.

In chapter 7 another example of autoantibodies directed towards modulated proteins is presented. Antibodies themselves can be cleaved into Fab or F(ab’)2 fragments by inflammation associated proteases. The hinge region of these antibody fragments may serve as a neo–epitope and elicit an antibody response. Indeed anti–hinge antibodies (AHA) could be found in RA patients in different stages of the disease, including the preclinical stage. Interestingly, antibodies directed towards the hinge of IgG4 antibodies (AH4A) were the most specific for RA as compared to AHA to the hinge of other IgG subclasses. IgG4 antibodies cannot activate complement and are regarded as tolerogenic. However, AH4A in complex with IgG4 F(ab’)2 fragments were able to activate complement, which indicates that AH4A can antagonize the anti–inflammatory properties of IgG4.

As such, next to the other antibodies, AH4A may play a role in initiating and perpetuating the vicious ‘cycle of RA’. This cycle is initiated by some unknown trigger that causes subclinical inflammation. Against a genetically susceptible
background, this inflammation causes modulation of antigens by various routes to which new antibodies can be formed. These antibodies can form complexes with their antigens and each other (in case of RF and AHA) and activate complement, further enhancing and perpetuating inflammation.

This work supports the hypothesis that the cycle of RA is already initiated years before RA is diagnosed. At a preclinical stage the deregulation of the immune system already shows some characteristics of a chronic inflammation. At the moment of diagnosis, the immune response is mature and interventions at this stage can only dampen the inflammation, but not cure the disease. The recognition of a preclinical stage gives hope of prevention of chronic disease by intervening much earlier in the disease process. However what this preclinical stage is exactly and which individuals can be regarded as having preclinical disease is not yet totally clear. It remains a challenge to accurately predict which patients will develop RA.

**CLINICAL PARAMETERS AND THE PREDICTION OF RA**

To investigate whether and how arthritis development can be predicted more precise than with antibody status alone, we followed a cohort of patients with arthralgia and ACPA and/or RF (seropositive arthralgia) for the development of arthritis. The value of various clinical parameters in the prediction of arthritis was investigated.

In chapter 8, it is reported that a single measurement of acute-phase reactants seems to be of limited value for prediction of development of arthritis in individual patients. Although some of the acute phase biomarkers showed a trend toward higher levels in those who later developed arthritis, these trends did not reach statistical significance. These results are in contrast with previous studies, in which CRP and SPLA2 levels were significantly elevated in preclinical RA. In contrast with previous studies, in which CRP and SPLA2 levels were significantly elevated in preclinical RA.30,31 However, these levels were still within the normal range and therefore large numbers of samples are needed to detect such small differences. Furthermore, CRP levels of patients with arthralgia were higher than CRP levels of healthy controls, indicating that patients with arthralgia have a slightly activated acute-phase response on the group level, although within this group an elevated acute-phase response was not predictive for the development of arthritis.

The presence of activated type I interferon (IFN) response genes on the other hand does predict arthritis development, as is reported in chapter 9. The activation of type I IFN response genes was measured in full blood mRNA of seropositive
arthralgia patients with microchip assays. A type I IFN score was calculated on the basis of the expression of six response genes: *IFI44L, IFI6, IFIT1, MXA, OAS3, RSAD2* and *EPSTI*. Patients with a high IFN score had a higher risk of developing arthritis than patients with a low score, independent of ACPA and RF status. These results were confirmed in pre-disease samples from blood donors who later developed RA. Since current technologies allow accurate, easy and cheap measurement of transcript quantification, the measurement of the IFN score could be a valuable addition to the measurement of antibodies in clinical practice, especially in patients that have an intermediate risk.

That also lipid levels are associated with future arthritis development is described in chapter 10. A lower ApoA1 level was associated with the development of arthritis even after adjustment for ACPA status. Dyslipidaemia and also an increased risk of cardiovascular disease were already known to be associated with RA. With this study we show that at least dyslipidaemia is present before diagnosis of RA. Whether or not an increased risk of cardiovascular disease is also present before onset of disease, remains to be investigated.

In chapter 11, the value of ultrasonography (US) in predicting arthritis in seropositive arthralgia patients is reported. A significant association between US abnormalities and arthritis development was detected at joint level. However, this association did not reach statistical significance at patient level, although a trend was still present. Patient numbers may have been too small and prevalence of abnormalities to low to detect significant associations. Furthermore, US was only performed once. Since subclinical arthritis as detected by US might have a waxing and waning character, a single measurement may not be enough to detect persistent subclinical arthritis with certainty. Further research is necessary to improve test characteristics. US may then prove to be a useful tool to predict arthritis development.

Finally, in chapter 13, a model is presented to predict the development of arthritis. Out of 18 variables the strongest predictors were selected to create a prediction model. Interestingly, though not totally unexpected, one of the resulting prediction variables was ‘not consuming alcohol’. The details of this finding were further analyzed and are reported in chapter 12. Alcohol consumption seemed to protect against arthritis development and this immunosuppressive effect may have partly been mediated via decreased SE expression.

Eight other prediction variables; a first degree relative with RA, arthralgia < 12 months, symptoms in both upper and lower extremities, VAS pain ≥ 50, morning
stiffness, a swollen joint as noticed by the patient and antibody status, made up the rest of the model. All prediction variables were assigned weighted scoring points resulting in a simplified prediction rule. With this rule, patients could be categorized into three risk groups; low, intermediate and high. After five years, 12% of the low risk patients, 43% of the intermediate risk patients and 81% of the high risk patients developed arthritis. This shows that with this rule it is possible to predict the development of arthritis. Since no complicated or expensive tests are necessary, the rule can readily be used in clinical practice, although further validation in other cohorts is still necessary.

**CONCLUDING REMARKS AND IMPLICATIONS FOR FUTURE RESEARCH**

From the work in this thesis it can be concluded that RA does not start at the moment that it is diagnosed. Rather, at the moment RA is diagnosed, the disease has already matured to its full inflammatory potential and the symptoms that are clinically apparent are the result of an inflammatory process that has been progressing for years. In some arthralgia patients this process has progressed considerably and these patients share many characteristics with patients with established RA, for instance an extended ACPA response and the presence of other autoantibodies. As such, the disease seems to be a continuum in which inflammation may have a waxing and waning character but has an overall crescendo trend. When a certain threshold is reached, arthritis may be detected and patients can be diagnosed with RA. In whom RA will develop can be predicted with some of the characteristics shared by RA and arthralgia patients.

There are many more features associated with RA that were not studied in this thesis in the context of the development of RA. For instance, it would be interesting to investigate the genetics of seropositive arthralgia patients in more detail. The DERAA hypothesis, which suggests that major histocompatibility complex (MHC) class II molecules harboring the DERAA motive are protective against RA,\textsuperscript{35} is of special interest in these patients.

Another interesting field is that of infectious diseases. Periodontitis is associated with RA and at a pathophysiological level shares many characteristics with RA.\textsuperscript{16-39} The bacterium involved in periodontitis, Porphyromonas gingivalis, is known to express PAD, the enzyme that citrullinates proteins.\textsuperscript{40} Therefore, an infection with this bacterium might trigger the formation of ACPA.\textsuperscript{41-43} It would be interesting to see whether periodontitis plays a role in the development of arthritis and
prospectively followed seropositive arthralgia patients can form the ideal cohort to test this hypothesis.

Cardiovascular diseases are also highly associated with RA.\textsuperscript{44-48} The causality of this association remains a matter of debate. RA and cardiovascular diseases might be the result of a genetic background shared by both, they might result from the same inflammatory process or one might be the result of the other via the enhanced inflammation caused by the latter. In this respect, studying cardiovascular risk factors and diseases in arthralgia patients seems an important research field.

Other subjects that deserve attention are the pathogenicity of autoantibodies and the involvement of the cellular component of the immune system in a very early stage of disease. What makes antibodies in RA different from other antibodies remains an intriguing question. Maybe their glycan profile is involved.\textsuperscript{49} Whether these antibodies are truly pathogenic is another matter of debate. Now that we have developed a tool to clone monoclonal antibodies out of patients, a way is opened to come closer to an answer. Yet another unanswered query is whether the cell types involved in established RA are the same as in arthralgia patients. We now have the means to put this question to the test. We can select patients with a high risk of developing RA and characterize their inflammatory profile in close detail and follow it over time.

Patients with high risk can also be selected for clinical trials. The ultimate goal for future research obviously remains the prevention of RA. A previous study showed that two intramuscular dexamethason injections did not prevent RA, although it led to a significant decrease in ACPA and RF levels.\textsuperscript{50} Other options are the use of traditional DMARDs or biologicals. At present a multicentre, double blind, placebo controlled trial studying the preventive ability of rituximab in high risk patients is being performed. This seems a good option since rituximab depletes B-cells by binding CD20 and has been shown to give a rapid and sustained clinical remission with a single treatment in RA patients.\textsuperscript{51}

An approach directed at T-cells instead of B-cells is the use of abatacept (CTLA4–Ig). This soluble fusion protein consists of the extracellular domain of CTLA4 and the Fc portion of IgG1. It binds CD80 (B7−1) and CD86 (B7−2) on antigen presenting cells, thereby acting as a competitive inhibitor of the CD28–B7 costimulatory interaction, preventing the second activation signal received by T cells via CD28.\textsuperscript{52,53} This biological is also proven to be effective in treating RA patients.\textsuperscript{54} Theoretically, it interacts with the inflammatory pathway ‘upstream’ of rituximab and therefore may be more effective in preventing the development of RA.
Abatacept may also act on tolerance induction, since CTLA4 is expressed by regulatory T cells and defects in CTLA4 are associated with abnormal Treg function in RA.\textsuperscript{55} Restoring tolerance seems the ideal strategy to prevent RA, since breakdown of tolerance already occurs before RA becomes clinically visible and auto-antibodies are a hallmark of RA. In animal models tolerance induction has been proven possible by oral administration of different proteins and peptides.\textsuperscript{56-65} In humans, the results of three double blind trials in tolerance induction have been published that compare the effect with oral collagen to placebo in RA. In these studies small but significant effects were seen on disease activity.\textsuperscript{66-68} Unfortunately, the therapeutic window was small (smaller or larger doses had no effect) and the magnitude of the effect was comparable to that of non steroid anti-inflammatory drugs. Treatment with classical DMARDs and biologicals remains much more successful. However, in the prevention of RA, tolerance induction could still play a role, since in animal studies tolerance induction before immunization seems more effective than after immunization.\textsuperscript{69} Furthermore, tolerance induction could be combined with traditional DMARDs since a synergistic effect was found between oral tolerance and methotrexate in animal studies.\textsuperscript{70,71} Combining tolerance induction with a biological interacting with tolerance mechanisms such as abatacept might prove even more effective. In addition, there are other antigen specific approaches to induce tolerance, such as cell based treatments with for instance tolerogenic dendritic cells.\textsuperscript{72} These seem elegant strategies to prevent the development of RA,\textsuperscript{72} albeit that ‘the specific antigen’ in RA is still unknown.

In conclusion, the studies presented in this thesis add to the notion that the evolvement of RA is an intricate process that may take several years and happens before the disease is diagnosed. The development of RA can be predicted to a certain degree, and further research may fine tune this prediction. Individuals with a high risk of RA might benefit from preventive treatment. Multiple strategies to prevent RA are available, but the value of these strategies remains to be investigated. The prediction model we have developed can help to select the appropriate patients to enroll in such studies.
REFERENCES


Summary and Discussion


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