Chapter 1

Introduction

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and

Anti-citrullinated protein antibodies as a risk factor for rheumatoid arthritis.

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RHEUMATOID ARTHRITIS

Rheumatoid arthritis (RA) is a disease characterized by arthritis of mainly the small joints of the hands and feet, which is thought to be the result of an autoimmune response. It is the most common inflammatory arthritis with a prevalence of 0.5-1.0% in European and North-American populations [1]. There is substantial geographic variation in the occurrence of RA with very high prevalences reported in native American-Indian populations [2], and very low prevalences in populations from South-East Asia [3]. The disease is approximately three times more frequent in women than in men, and the prevalence increases with age. Besides the potentially destructive arthritis, patients can be affected by various extra-articular features such as secondary Sjögren's syndrome, interstitial lung disease, pericarditis and pleuritis. Fortunately, the advent of tumor necrosis factor (TNF) inhibitors and other biological agents have led to a therapeutic revolution for patients with rheumatoid arthritis [4]. Instead of having to resign to an inevitably progressive and debilitating disease course, modern-day treatment aims at achieving the lowest possible disease activity and ultimately remission. Nonetheless, rheumatoid arthritis continues to be a major cause of (partial) disability and of loss of productivity, and is associated with substantial economic costs [5].

Classification criteria for the disease were first phrased in 1956 [6] after Sir Alfred Garrod had introduced the term rheumatoid arthritis in 1876 in an attempt to counteract the unsatisfactory use of designations such as "chronic rheumatism" and "rheumatic gout" [7]. The purpose of the classification criteria was to facilitate both clinical diagnosis and scientific research. For many years since, the 1987 American College of Rheumatology (ACR) classification criteria have been used to this end, despite the fact that the incorporation of items such as erosive radiographic changes led to limited diagnostic value of these criteria for patients with early arthritis [8]. In order to facilitate the study of persons with earlier stages of disease, the ACR and the European League Against Rheumatism (EULAR) have recently developed the 2010 classification criteria for RA as shown in Table 1 [9]. It is worthwhile to note that these criteria are based on patient characteristics which were associated with the decision by the physician to start treatment with methotrexate. These criteria are a reflection of the shift towards increasingly earlier diagnosis and treatment of rheumatoid arthritis.

AUTOANTIBODIES IN GENERAL AND ANTI-CITRULLINATED PROTEIN ANTIBODIES (ACPA) IN PARTICULAR IN RHEUMATOID ARTHRITIS

Rheumatoid factor (RF) is the classic autoantibody associated with RA. It has long been known to be a marker of future RA development [10]. However, the predictive ability of

Table 1. The 2010 ACR/EULAR classification criteria for rheumatoid arthritis

Target population: patients
1) who have at least one joint with definite clinical synovitis (swelling)
2) whose synovitis is not better explained by another disease.

Criteria	Score
A. Joint involvement:	
- 1 large joint	0
- 2-10 large joints	1
- 1-3 small joints (with or without involvement of large joints)	2
- 4-10 small joints (with or without involvement of large joints)	3
- > 10 joints (at least 1 small joint)	5
B. Serology (at least one test result is needed for classification):	
- Negative RF *and* negative ACPA	0
- Low-positive RF *or* low-positive ACPA	2
- High-positive RF *or* high-positive ACPA	3
C. Acute-phase reactants (at least one test result is needed for classification):	
- Normal CRP *and* normal ESR	0
- Abnormal CRP *or* abnormal ESR	1
D. Duration of symptoms:	
- < 6 weeks	0
- ≥ 6 weeks	1

A score of ≥ 6/10 is needed for classification of a patient as having definite RA.
Joint involvement refers to any swollen or tender joint on examination. Distal interphalangeal joints, first carpometacarpal joints and first metatarsophalangeal joints are excluded from assessment. RF: rheumatoid factor. ACPA: anti-citrullinated protein antibody (tested as anti-cyclic citrullinated peptide (anti-CCP), CRP: C-reactive protein, ESR: erythrocyte sedimentation rate.

immunoglobulin M (IgM)-RF is limited by its relatively modest specificity (85%), which entails that many patients who are IgM-RF-positive will not develop RA [11]. Recently, a better diagnostic and predictive marker has emerged in the form of anti-citrullinated protein antibodies (ACPA).

ACPA bind to proteins or peptides containing citrulline, which is a post-translational modification of the naturally occurring amino acid arginine. By an enzymatic process called deimination or citrullination which is mediated by a series of enzymes known as peptidyl arginine deiminases (PADs), the positively charged peptidylarginine is converted into the neutral peptidylcitrulline (Figure 1) [12]. The activity of PAD enzymes is dependent on high concentrations of calcium, and deimination can occur both intracellularly concurrent with apoptosis, and extracellularly provided the calcium concentration is high enough [13, 14]. Besides the occurrence of endogenous citrullination, recent reports have drawn attention to the possible role of the oral bacterium Porphyromonas gingivalis (P. gingivalis) which also posses PAD [15]. This pathogen is the major etiologic agent associated with periodontitis; a condition which is significantly increased in patients with longstanding active RA [16]. These associations raise the possibility that citrullination of bacterial and host proteins by P. gingivalis may be a mechanism by which citrullinated antigens in RA are generated.

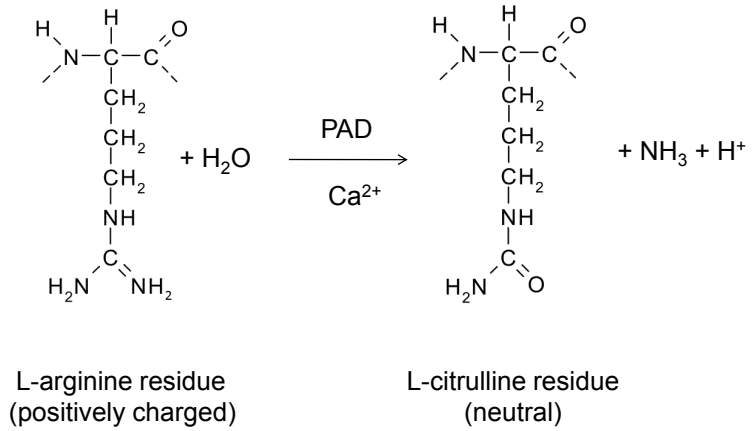

Figure 1. The enzymatic process of citrullination.

The exact function of citrullination is not fully known although it has been described to make proteins more prone to degradation by proteolytic enzymes [14]. The discovery that proteins undergoing processing in antigen-presenting cells (APCs) can be citrullinated before presentation of their peptides to T cells is immunologically interesting, although the exact consequences for the immune response remain unclear [17]. Citrullinated proteins have been detected in a variety of inflamed tissues, such as in inflamed joints in several different forms of arthritis, in lungs and other organs [18]. Furthermore, citrullination has been shown to take place in lining and sublining cells in the joints of patients with RA [13].

ACPA were first described as anti-perinuclear factor and anti-keratin antibodies, both of which bind to filaggrin [19-21]. It was not until several years later that recognition of this antigen was found to be exclusively dependent on the presence of citrulline residues [22]. The same was the case for citrullinated vimentin, which was discovered to be the target of the RA-specific anti-Sa antibodies that had been described many years before [23, 24]. Based on these findings, several commercial assays that test for the presence of antibodies to cyclic citrullinated proteins (CCP) have been developed and successfully introduced into clinical practice [25]. The commercial ACPA tests have been designed to detect most, if not all, ACPA epitope reactivities found in vivo. The test which is currently most widely used in clinical practice is the second generation anti-CCP test, although other targets such as mutated citrullinated vimentin are also employed [26].

DIAGNOSTIC AND PROGNOSTIC VALUE OF ACPA

A comprehensive overview of the diagnostic utility of the anti-CCP and RF tests in RA has been provided by a meta-analysis [11, 27]. The most important difference between the two tests was the increased specificity of the anti-CCP assays versus IgM-RF (95% vs. 85%). The sensitivity of anti-CCP2 was similar to that of IgM-RF (67% in patients with established rheumatoid arthritis). In patients with early rheumatoid arthritis (<2 years of disease) the summary sensitivity was 57% [28]. The diagnostic characteristics of IgG-RF and IgA-RF, which had previously been reported to be more accurate than IgM-RF, proved to be similar to IgM-RF; thus, these measurements do not provide additional information.

With regard to other rheumatologic diseases, ACPA have been detected in 5-15% of patients with psoriatic arthritis [29, 30] and in patients with juvenile idiopathic arthritis, in whom IgG-anti-CCP was most often present in IgM RF-positive polyarthritis which most closely resembles adult RA [31]. Anti-CCP antibodies have also been reported in patients with non-rheumatologic diseases such as pulmonary TB [32]. However, follow-up studies have revealed that sera from these patients also frequently react with unmodified arginine-containing peptide, suggesting that these antibodies are not citrulline-specific [33]. Of healthy controls, 1% tests positive for anti-CCP2-antibodies as was shown in an overview of 144 independent studies [34]. It is unclear how many of these people may develop RA in the future.

In view of these data, it is necessary to re-examine how serological markers can be used most effectively in the diagnostic strategy for RA. Determination of anti-CCP2 antibodies, which have a high specificity and a reasonable sensitivity, is a good first option in patients presenting with inflammatory arthritis. However, anti-CCP-assays are still considerably more expensive than measurements of RF in most countries. Whether it is worthwhile to also determine IgM-RF depends on the interpretation of the results. Requiring a positive result on both tests leads to a loss of sensitivity, because fewer patients have both antibodies than either RF or anti-CCP alone. On the other hand, considering a positive result on one test as sufficient for a diagnosis of RA would lead to a considerable reduction in specificity (especially when only using the RF test) in exchange for a small gain in sensitivity. This balance between sensitivity and specificity needs to be kept in mind when deciding whether to measure IgM-RF in addition to anti-CCP2.

The 2010 ACR/EULAR classification criteria for RA have included an extra level of complexity by discerning between low-positive and high-positive IgM RF and ACPA results, where low-positive refers to positive values ≤ 3 times the upper limit of normal (ULN), while high-positive refers to values >3 times the ULN (Table 1). Although it has previously been reported that the presence of high levels of IgM RF would lead to increased sensitivity for RA [35], a recent study showed that a high level of RF has very limited additive prognostic value compared to ACPA-positivity [36]. The 2010 criteria for RA therefore could

be improved by omitting the RF level criterion. There are no clear results with regard to the value of anti-CCP levels in this respect [37, 38].

During the disease course of RA, anti-CCP levels generally decline from the baseline value [39]. In a study investigating intensive treatment in early RA, anti-CCP levels were even reported to decrease by 50% [40]. In contrast to IgM-RF however, there is hardly any seroconversion, meaning that despite declining levels, patients who once tested anti-CCP-positive hardly ever become anti-CCP-negative [39, 40]. Conflicting results have been reported with regard to the association between anti-CCP levels and disease activity [41], although the majority of publications have described that a higher level of anti-CCP antibodies at baseline is associated with a higher level of disease activity, a less favorable response to treatment with methotrexate and increased joint destruction [42-44]. Two small longitudinal studies have investigated whether anti-CCP levels fluctuate in parallel with disease activity and were not able to draw definitive conclusions [45, 46]. Larger scale investigations of whether anti-CCP level fluctuations may occur prior to or concomitantly with an increase in disease activity are lacking.

In addition to their function as a diagnostic marker for RA, ACPA have also been extensively investigated with regards to their long-term predictive abilities. The presence of ACPA is currently the best predictive marker for the progression of undifferentiated arthritis (UA) to rheumatoid arthritis (RA). In multivariate logistic regression analysis that also accounted for other predictive variables, anti-CCP2 positivity was associated with an odds ratio of 8.1 (95% confidence interval 4.2–15.8; $p<0.001$) for the development of RA in UA patients [47]. ACPA-positive RA patients also have a more severe disease course than ACPA-negative RA patients, with more joint destruction and extra-articular manifestations of disease [43, 48, 49].

Several studies have investigated the time-point at which patients develop ACPA. By making use of pre-disease samples from blood-bank donors who later developed RA, these reports were able to demonstrate that ACPA can be detected many years before disease manifestation [50, 51] and that ACPA titers increase up to the point of disease onset.

The fact that ACPA appear during the preclinical phase of RA, together with the finding that ACPA can exacerbate arthritis in mice, suggest that anti-citrullinated protein immunity may play a role in the pathogenesis of the disease [52]. This has prompted investigations into which risk factors are associated with the emergence of the anti-citrullinated protein immune response.

GENETIC RISK FACTORS FOR RHEUMATOID ARTHRITIS

The risk of developing RA is known to be influenced by several genetic risk factors, of which the Human Leucocyte Antigens (HLA) are the most important. The association between RA and the HLA region was originally described more than 30 years ago [53], and subsequent studies revealed that several of the HLA-DR alleles are associated with RA, which led to the formulation of the Shared Epitope (SE)-hypothesis in 1987 [54]. This hypothesis provided a theoretical background for the observed associations between the HLA region and RA based on the fact that all HLA-DR alleles which predispose to RA have the same or a similar amino acid sequence (shared epitope) at positions 70-74 of the HLA-DRB1 molecule. This sequence is located in the peptide-binding groove of the HLA alleles and may therefore be directly involved in the presentation of peptides to arthritogenic T cells.

After the first descriptions of ACPA, it soon became clear that the SE alleles were solely associated with, and thus only predisposed to, ACPA-positive RA [55]. Several reports showing that certain HLA SE alleles can bind and present citrullinated peptides [56, 57], have provided a possible biological explanation for the association between ACPA and the SE alleles. A chemical analysis of an induced murine immune response to citrullinated collagen type II demonstrated that the conformational change in the antigen induced by citrulline was critical for recognition by antibodies [58], again suggesting that citrulline plays an essential role in the initiation of the immune response. Intriguingly, further research has now shown that the SE alleles mainly predispose to the development of ACPA, rather than to the development of RA [59]. This suggests a pathophysiological sequence of events detailed below in which genetic predisposition results in the development of anti-peptidylcitrulline immunity prior to the onset of RA.

After these seminal findings with regards to the association between the HLA SE alleles and RA, many of the genetic risk factors for RA have now been found to be specific for either ACPA-positive or ACPA-negative RA. The majority of genetic risk factors, such as PTPN22, the TRAF1-C5 locus, the OLIG3-AIP3-locus and STAT4, are preferentially associated with ACPA-positive RA [60, 61]. Conversely, there are other genetic risk factors that have been described to be exclusively associated with ACPA-negative RA, such as HLA-DR3 and interferon regulatory factor (IRF) haplotypes [62]. As there are no markers available that are specific for this disease subset, it is impossible to determine at present whether these genetic risk factors predispose to ACPA-negative RA or to specific immunological alterations in these patients.

ENVIRONMENTAL RISK FACTORS

In addition to genetic aspects, environmental risk factors are known to contribute to the etiology of RA. Many epidemiological studies have shown an association between cigarette smoking and the development of RA [63, 64]. Smoking was found to interact with the HLA SE alleles in the predisposition to RF-positive RA [65]. However, recent data have shown a striking interaction between smoking and the SE alleles in conferring risk for ACPA-positive, rather than for RF-positive RA [66]. They also demonstrated an association between smoking and the development of citrullinated antigens in bronchoalveolar lavage fluid cells, thereby providing a possible pathogenetic link between smoking and the development of ACPA-positive RA.

PATHOPHYSIOLOGICAL MODEL OF RHEUMATOID ARTHRITIS

The recent findings that have elucidated the differences in risk factors for ACPA-positive versus ACPA-negative RA have important consequences for the understanding of the pathophysiology of RA, which can be viewed as a multi-stage process (Figure 2).

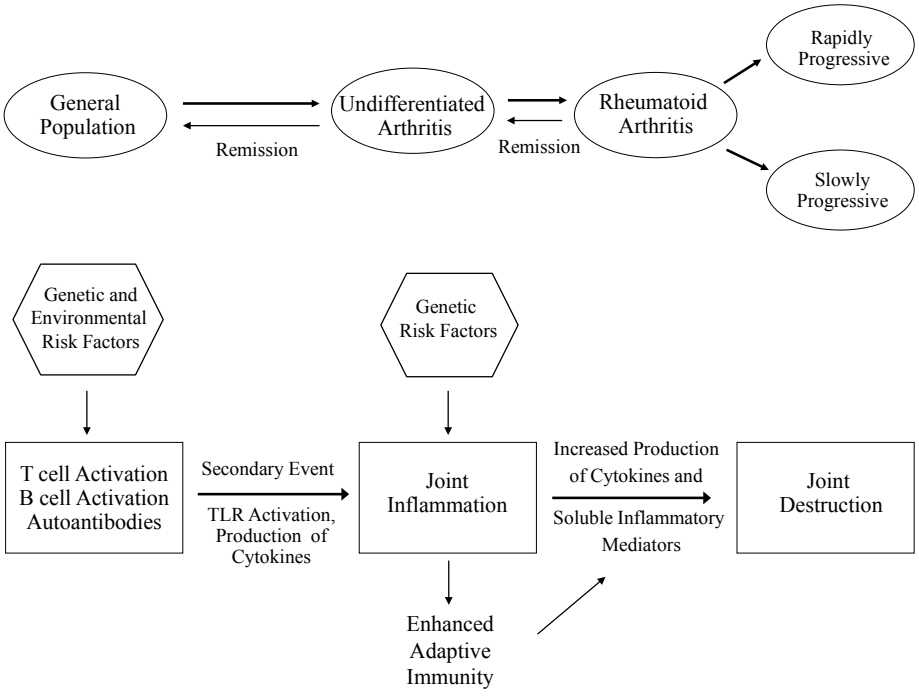

Figure 2: A multi-stage model of the clinical and pathophysiological development of RA.

In the first stage, a genetic predisposition along with environmental factors results in an adaptive immune response with antigen-dependent T and B cell activation. In the case of ACPA-positive RA, the SE alleles together with smoking may lead to the development of an anti-citrullinated protein immune response. A nonspecific environmental trigger could function as a secondary event leading to joint inflammation, which manifests itself as undifferentiated arthritis (UA). Other genetic risk factors involved in, for example, cytokine regulation, play a role in determining the extent and duration of joint inflammation. The inflammatory process itself can subsequently further stimulate the adaptive immune response through the generation of new epitopes by, amongst other processes, citrullination, which has been shown to occur more readily in inflamed joints. In individuals who have previously developed an adaptive immune response (possibly with production of ACPA), the immune cells that now gain access to the joints can enhance the inflammation and lead to the increased production of cytokines and soluble inflammatory mediators. Large numbers of activated CD4-positive T cells accumulate in the joints of patients with RA. The abundant presence of these activated T cells in the joints has several different effects. Through production of cytokines and through cell-surface interactions, the activated T cells stimulate monocytes, macrophages, and synovial fibroblasts to produce other cytokines such as TNFα and to secrete matrix metalloproteinases [67]. Furthermore, the activated T cells stimulate osteoclastogenesis [68]. Finally, the autoreactive T cells are capable of providing help to autoreactive B cells, which can lead to the production of autoantibodies. B cells can also function as antigen-presenting cells and thus can promote T cell activation and perpetuation of the autoimmune response [69]. Activated B cells and autoantibody-producing plasma cells are present in RA synovium, although it is not completely clear if the original B cell activation occurs in the joint or elsewhere. ACPA bound to citrullinated antigens have also been detected in the joints of RA patients [70, 71].

Autoantibodies may play a pathogenic role by fixing complement in the joint leading to the release of chemotactic factors such as C5a and the subsequent recruitment of inflammatory cells. Another mechanism by which immune complexes can contribute to joint damage is through the activation of monocytes and macrophages by binding to Fc-receptors on the surface of these cells. This could cause perpetuation of the synovial inflammation and progression of UA to RA and erosive disease. This pathophysiological model, based on the activation of adaptive immunity, currently seems most applicable to ACPA-positive RA.

OUTLINE OF THIS THESIS

There were three main aims of this thesis:

1. to quantify the heritability of ACPA-positive and ACPA-negative RA, and to elucidate the association of distinct genetic and environmental risk factors with both subsets of disease.
2. to characterize the immunological properties of ACPA and to investigate the association between ACPA characteristics and disease progression.
3. to identify predictive factors for the most favorable outcome of RA: sustained remission after discontinuation of disease-modifying anti-rheumatic drugs (DMARDs).

The thesis is divided into three parts corresponding to the three main aims.

Part 1 is dedicated to the investigation of the heritability of RA, and to the exact association of specific genetic risk factors (the HLA-DRB1 alleles) and an environmental risk factor (alcohol) with ACPA-positive and ACPA-negative RA.

As described above, it has been known for many years that the most prominent genetic risk factor for RA is situated at the HLA locus. The HLA-DRB1 alleles that confer the strongest predisposition for RA are also known as the shared epitope (SE) alleles and have been found to selectively predispose to ACPA-positive RA. Other HLA-DRB1 alleles confer protection against disease although, due to conflicting results, it was unclear exactly which alleles are protective [72-74]. It was also unknown whether these protective effects might be limited to ACPA-positive or ACPA-negative RA. We therefore performed a meta-analysis of four European populations to investigate precisely which HLA-DRB1 alleles are associated with protection in ACPA-positive and ACPA-negative RA (**chapter 2**).

In addition to the HLA alleles, multiple other genetic risk factors have been described which predispose to RA. It is remarkable that the majority of these genetic risk factors have been found to preferentially confer risk to autoantibody-positive disease. This suggests that the extent to which genetic factors contribute to disease development may differ between ACPA-positive and ACPA-negative RA. In **chapter 3**, we therefore determined the heritability of ACPA-positive and ACPA-negative RA by applying quantitative genetic methods to data from a large twin cohort. Furthermore, we assessed the contribution of the predisposing HLA-DRB1 shared epitope (SE) alleles to the genetic variance of both groups.

Surprisingly, these studies described in chapter 3 revealed that that the heritability of ACPA-positive and ACPA-negative disease does not differ, and is 66% for both subsets of disease. The effect of the HLA SE alleles explained a relatively modest 18% of the genetic variance of ACPA-positive RA, raising the question which other genetic risk factors could account for the remaining 82% of "missing heritability". In light of the findings of chapter 2, which revealed a strong protective effect of HLA-DRB1*13 alleles for ACPA-positive disease, we investigated to what extent these protective HLA effects contribute to the

genetic variance of RA in **chapter 4**. Since a multitude of new genetic risk factors for RA in the form of single nucleotide polymorphisms (SNPs) has been described in the past few years, the effect of 12 well-replicated SNPs was also taken into account.

Besides genetic risk factors, there are also environmental risk factors which are known to predispose to RA, such as smoking. A recent case-control study proposed that another environmental risk factor: alcohol intake, may be associated with a lower risk of developing RA [75]. This is in line with findings from experimental studies showing a downregulatory effect of alcohol on both innate and adaptive immune responses [76-78]. In **chapter 5** we evaluated whether the protective effect of alcohol is specific for RA, or may extend to other forms of inflammatory arthritis as well. Furthermore, we assessed if the protective effect differed between the ACPA-positive and ACPA-negative subset of disease.

Part 2 focuses on the immunological characteristics of ACPA, the influence of genetic and environmental risk factors on the shaping of the ACPA response, and the clinical value of the various commercially available ACPA tests.

Different parts of human antibodies have different functions. While the antigen specificity is determined by the Fab (Fragment antigen binding) region, the Fc (Fragment crystallizable) region is responsible for immune effector functions such as complement activation, antibody-dependent cellular cytotoxicity and binding to cellular Fc-receptors. These Fc-mediated effects are influenced by Fc-linked carbohydrate structures known as glycans. By glycosylation, a posttranslational modification process, different kinds of glycan structures can be attached to the Fc-parts of the antibodies, resulting in several different glycoforms in human serum [79]. Interestingly, early studies have demonstrated a predominance of IgG-G0 (meaning without galactose) glycoforms in sera of RA patients which correlated with disease activity and reverted to normal levels in patients who achieved (spontaneous) remission [80, 81]. To investigate whether ACPA, which are hypothesized to play a crucial role in the pathogenesis of RA, carry a different glycosylation pattern than other human antibodies, we developed a technique that allowed analysis of Fc-linked glycans in an antigen specific manner [82]. **Chapter 6** describes the results of the analysis of the ACPA glycan profile from serum and synovial fluid.

Human antibodies consist of four polypeptide chains: two heavy chains and two light chains. There are five different types of heavy chains that determine the isotype of the antibody: IgM, IgD, IgG_{1-4}, IgA and IgE. The isotypes differ substantially with regard to their ability to mediate effector mechanisms, such as complement activation and binding to Fc-receptors. The ACPA response has been shown to consist of various isotypes, and the fact that ACPA of the IgM isotype could be detected at various stages of the disease indicated that the anti-citrullinated protein immune response is constantly being (re)activated [83-86]. It was unclear however, if during this process of continuous reactivation throughout disease progression, there was also further maturation of the ACPA response in the sense of isotype switching. In addition, the finding that the ACPA isotype use differed between

patients [86], raised the question whether changes in isotype use are associated with disease progression. In **chapter 7**, we therefore examined the relationship between ACPA isotypes and disease development and progression. The use of two large, independent cohorts with long-term follow-up data enabled us to investigate the association between the ACPA isotype profile and progression of radiographic damage.

Another antibody characteristic which is crucial for the pathogenicity of autoantibodies, is the fine specificity of antigen recognition [87]. The important pathophysiological consequences of an increase or shift in antigen recognition during the course of an immune response (a phenomenon known as epitope spreading), have been described in for example systemic lupus erythematosus (SLE) [88]. Knowing that ACPA can be detected before the clinical diagnosis of RA, we hypothesized that similar to SLE, epitope spreading of the ACPA response may play a role in the evolution of the disease. We therefore investigated the reactivity pattern of ACPA before disease onset and during disease progression, as described in **chapter 8**. Making use of several different cohorts, which cover the entire spectrum of the disease course ranging from ACPA-positive healthy individuals to long-standing RA, this study provides an overview of the ACPA fine specificity development over time. The clinical effects of individual differences in ACPA fine specificity repertoire were examined in more detail in **chapter 9**. Based on the known association between the HLA SE alleles and some ACPA fine specificities as described below, this association was now investigated with regard to several more citrullinated epitopes. Moreover, an in-depth analysis of the effect of SE gene dose was performed. In order to investigate the clinical outcome of differences in the ACPA fine specificity repertoire, we investigated whether certain ACPA fine specificities were associated with the extent of radiographic joint damage.

As mentioned in the first part of this introduction, the HLA SE alleles and smoking are known to predispose to the development of ACPA-positive RA. The combined effect of HLA SE alleles and smoking has been shown to exceed the sum of their single effects: a phenomenon known as biological interaction [89-91]. Furthermore, a previous study on ACPA fine specificity reported that the HLA SE alleles may also determine the fine specificity of the ACPA response, since they predisposed to the development of antibodies against citrullinated vimentin but not against citrullinated fibrinogen [92]. Based on these results, we wondered whether genetic and environmental risk factors might also interact with regard to their effect on the fine specificity of ACPA. If interaction were to be found in association with ACPA-reactivity to a particular citrullinated protein, this may indicate that the protein under investigation is of etiologic importance as an autoantigen in RA. Partly in response to a publication postulating a specific interaction between genotype, smoking and autoimmunity to citrullinated α-enolase peptide (CEP-1) [93], we analyzed the effects of HLA SE alleles and smoking on the ACPA reactivity profile in **chapter 10**. The results of this analysis revealed that the interaction between genotype, smoking and autoimmunity to

citrullinated antigens was not confined to citrullinated α-enolase, but extended to several other citrullinated antigens as well.

Chapter 11 provides additional essential information with regard to these results. We hypothesized that the gene-environment interaction between the HLA SE alleles and smoking affected the development of ACPA-positive RA in general, rather than the development of specific ACPA fine specificities. When the analysis was performed within ACPA-positive patients, the interaction was no longer present, which argues in favor of our hypothesis, and against an effect of the gene-environment interaction on shaping the reactivity of the ACPA response.

While the ACPA fine specificity studies mentioned above were all performed with in-house assays specifically developed for this purpose, we also examined the clinical utility of the ACPA tests which are commercially available in **chapter 12**. The aim of this investigation was to compare the predictive ability of anti-CCP2-, anti-CCP3-, anti-modified citrullinated vimentin (anti-MCV-) and RF-tests with regard to three outcome measures: progression from UA to RA, the rate of joint destruction and achieving sustained DMARD-free remission in RA.

Part 3 of this thesis describes the results of two studies concerning remission in rheumatoid arthritis.

RA patients vary considerably in terms of their disease course and outcome. The spectrum extends from debilitating, destructive joint disease on one side, to remission, the most favorable outcome, on the other side [94]. These days, remission is increasingly becoming an attainable goal of RA treatment, especially since the introduction of biological anti-rheumatic therapy. The potent suppression of disease activity which can be achieved with these novel treatment agents, together with the finding that early aggressive therapy leads to better long-term outcomes, has resulted in new treatment goals striving to achieve remission in as many patients as possible [95, 96].

Remission rates after treatment with new therapeutic agents are now often reported, but there are few data on remission after treatment with conventional therapy, such as non-steroidal anti-inflammatory drugs (NSAIDs) and non-biological disease modifying anti-rheumatic drug (DMARD) therapy. These figures are however required for a fair comparison of the remission rates reported in clinical trials of novel agents. Moreover, the investigation of remission as a definitive disease outcome resembling cure is very important from a pathophysiological point of view. Data on patient characteristics associated with remission could lead to new hypotheses about the biological pathways involved in disease persistence and resolution. In **chapter 13**, we therefore used two large independent cohorts of patients treated with conventional therapy to examine the prevalence and predictive factors for disease modifying anti-rheumatic drug (DMARD)-free sustained remission.

The results of this study were extended in a subsequent investigation in which we compared the prevalence and predictive factors for remission in two different patient cohorts. Conventional therapy as mentioned above, was used in one of the cohorts, while the other cohort was treated according to a tight control strategy. Tight control entails a treatment strategy aiming at a predefined goal of minimal disease activity. This strategy has been shown to lead to improved functional status and high remission percentages, irrespective of the exact initial choice of therapy [97]. In **chapter 14**, we investigated whether this tight control strategy aiming at a low disease activity score (DAS < 1.6) resulted in more drug-free remission, or led to drug-free remission in patients who were more difficult to treat.

Chapter 15 provides a summary of the results and a discussion of the implications of the studies described in this thesis.

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