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Title: The influence of autoantibody status and characteristics on the course of rheumatoid arthritis
Issue Date: 2014-06-05
CHAPTER 1

General introduction

Adapted from:

The influence of ACPA status and characteristics on the course of RA.

*Nat Rev Rheumatol.* 2012 Jan;8(3): 144-52

and

New Biomarkers in rheumatoid arthritis.

*Neth J Med.* 2012 Nov;70(9): 392-9
RHEUMATOID ARTHRITIS

Rheumatoid arthritis (RA), one of the most common autoimmune diseases, is characterized by persistent synovitis, systemic inflammation and expression of autoantibodies. In industrialized countries approximately 0.5-1.0% of the adult population is affected by the disease. There is substantial geographic variation in the occurrence of RA with very high prevalences reported in native American populations, and very low prevalences in populations from South-East Asia. The disease is approximately three times more frequent in women than in men, and the prevalence increases with age. As RA is a systemic disease, symptoms as fatigue, weight loss and fever as well as disorders of the heart, blood vessels, nerves and kidneys are also relatively common. The disease reveals itself by joint swelling and joint tenderness, in which the small joints of the hands and feet are most commonly affected. The persistency of synovitis can result in the destruction of cartilage and subchondral bone, eventually leading to malformations and disability. If left unattended or not properly treated, RA can lead to increased disability or even invalidity of patients in their normal daily functions, thereby reducing the quality of life. For society this can ultimately lead to enormous costs in healthcare and loss in workforce.

The mainstay of treatment in RA, are the Disease Modifying Anti Rheumatic Drugs (DMARDs), which are a heterogeneous collection of therapeutic agents of which the mechanisms of action are, largely, not well understood. When arthritis stays uncontrolled despite these agents, or when toxic effects arise upon administration of these drugs, biologic agents, such as tumor necrosis factor inhibitors can be used and have proven to be highly effective. RA is considered as having an autoimmune origin because of the presence of self-reactive antibodies, such as anti-citrullinated protein antibodies (ACPA), thereby reflecting the complexity of the disease. Although it poses a considerable health problem, relatively little remains known about the disease pathogenesis and etiology.

The first description of RA was made in 1800 in Paris. Sixty years later, the disease was named ‘rheumatoid arthritis’ for the first time by an English rheumatologist, Alfred Baring Garrod. As RA seems to be a highly heterogeneous disease, several classification criteria have been developed over the years, such as the 1956 ARA criteria, 1961 Rome criteria and 1966 New York criteria, with the aim of identifying more homogenous patient groups to facilitate comparison of international studies. Since 1987 the disease has been classified based on the ACR 1987 criteria, which were developed using an analytical approach in which RA was defined by a regression analysis of disease characteristics of ‘classic cases’. As different disease manifestations are included in these criteria, a heterogeneous set of patients with conceivably different ‘pathogenic’ backgrounds are identified by them. Therefore, the great variation in disease course and treatment response
among patients with RA can be explained by the classification criteria describing a heterogeneous syndrome. ACPA have an exquisite specificity for RA\(^9\), and ACPA status was included in the new 2010 ACR–European League Against Rheumatism (EULAR) classification criteria for RA\(^{11,12}\), alongside RF levels (already included in the 1987 criteria) (figure 1).\(^9\)

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<thead>
<tr>
<th>ACR 1987 criteria</th>
<th>ACR/EULAR 2010 criteria</th>
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<tr>
<td>Morning stiffness (at least 1h)</td>
<td>Score</td>
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<tr>
<td>Arthritis of three or more joint areas</td>
<td>1 large joint</td>
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<td>Arthritis of hand joints (&gt;1 swollen joints)</td>
<td>2-10 large joints</td>
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<td>Symmetric arthritis</td>
<td>1</td>
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<tr>
<td>Rheumatoid nodules</td>
<td>1-3 small joints (large joints not counted)</td>
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<td>Serum rheumatoid factor</td>
<td>4-10 small joints (large joints not counted)</td>
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<td>Radiographic changes (erosions)</td>
<td>&gt;10 joints (at least one small joint)</td>
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<th>Serology</th>
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<td>Negative RF and negative ACPA</td>
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<td>Low-positive RF or low-positive ACPA</td>
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<td>High-positive RF or high-positive ACPA</td>
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<td>Acute-phase reactants</td>
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<td>Normal CRP and normal ESR</td>
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<td>Abnormal CRP or abnormal ESR</td>
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<tr>
<td>Duration of symptoms</td>
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<td>&lt;6 weeks</td>
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<td>≥6 weeks</td>
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A score of ≥6 is the cutpoint for rheumatoid arthritis. Patients can also be classified as having rheumatoid arthritis if they have: 1) erosive disease typical for rheumatoid arthritis, 2) long-standing disease previously satisfying the classification criteria.

**Figure 1. ACR 1987 criteria and ACR/EULAR 2010 criteria.** Classification criteria for rheumatoid arthritis. ACR = American College of Rheumatology. EULAR = European League Against Rheumatism. RF = rheumatoid factor. ACPA = Anti Citrullinated Peptide Antibodies. CRP = C-reactive protein. ESR = erythrocyte sedimentation rate. Adapted from Scott et al.\(^1\)

**AUTOANTIBODIES IN RA**

Various autoantibodies have been described in RA, including antibodies against ribosomal proteins (anti-RA33 antibodies)\(^{13,14}\) and against carbamylated proteins (anti-CardP antibodies).\(^{15}\) The first RA-associated antibody, rheumatoid factor (RF), was known by 1940\(^{16}\), and was later found to be directed to the Fc region of IgG. More than 20 years later, Nienhuis et al.\(^{17}\) described antibodies labeling perinuclear granules in the superficial cells of the human buccal mucosa epithelium, which were named anti-perinuclear factor antibodies (APF). Although it became clear that APF were highly specific for RA, testing for these antibodies was rather cumbersome; therefore, RF was assessed in daily practice. In 1979, anti-keratin
antibodies (AKA) were described, and found to be present in approximately 50% of patients with RA.\(^1\)\(^8\) Again, although these antibodies were more specific than RF for RA, the presence of RF was much more convenient to visualize. It was not until 1995 that the protein filaggrin was shown to be the common antigen targeted by both APF and AKA.\(^1\)\(^9\) Since then, research into this unique autoantibody system has taken off with unprecedented speed, reaching a climax early in 2011 with the inclusion of anti-citrullinated protein antibodies (ACPA) in the new classification criteria for RA.\(^1\)\(^1\), \(^1\)\(^2\)

Testing for ACPA

After the discovery of filaggrin as the target of APF and AKA, it took only a year to reveal the molecular identity of the antigens that these autoantibodies recognize: the nonclassical amino acid citrulline, embedded on a protein backbone.\(^2\)\(^0\), \(^2\)\(^1\) Citrulline is a non-encoded amino acid, generated by a post-translational modification of arginine mediated by protein-arginine deiminase enzymes (figure 2).\(^2\)\(^2\) This modification takes place during a variety of biological processes, including inflammation. In 1998, the first ELISA using citrullinated peptides (derived from several filaggrin epitopes) was developed\(^2\)\(^1\) and was followed, in 2000, by the first ELISA based on artificial cyclic citrullinated peptides (CCP).\(^2\)\(^3\) The first commercial version of this test, the CCP2 assay, became available in 2002 and enabled routine testing for antibodies directed against citrullinated epitopes as a biomarker for RA.\(^2\)\(^3\)-\(^2\)\(^7\) As well as the CCP2 assay, a few other assays for ACPA, such as CCP3 and MCV (Mutated Citrullinated Vimentin), have made their way into the clinic. These assays differ slightly in terms of specificity and sensitivity.\(^2\)\(^8\)

![Figure 2. Citrullination of an amino acid.](image)

Posttranslational modification of arginine into citrulline, mediated by peptidylarginine deiminase (PAD).
ACPA: cause or consequence?

The association with RA

ACPA are strongly associated with RA, which suggests they have a prominent role in disease pathogenesis. Indeed, it has been suggested that ACPA are the ‘spark that lights the RA fire’, and are directly involved in a vicious circle that explains the chronicity of RA. The efficacy of selective B-cell depletion in the treatment of RA provides evidence for the involvement of B cells and possibly autoantibodies in its pathogenesis. Furthermore, most ACPA-positive patients with RA seem to be ACPA-positive years before the onset of disease, although the extent of the ACPA armament seems to be limited at this preclinical stage. Moreover, ACPA levels show an increase around 2 years before the onset of symptoms, after which they seem to stabilize at fairly high levels.

Histological differences in inflamed joints have been found between ACPA-positive and ACPA-negative patients with RA. For example, synovial tissue immune cell infiltrates differ with respect to lymphocyte numbers as well as markers of fibrosis; altered synovial inflammatory architecture might indicate a role for ACPA in synovial inflammation.

Interaction of the ACPA and RF responses

Although the appearance of ACPA in the preclinical phase tends to precede that of RF, RF can also be detected years before clinical disease onset. RF seems to preferentially interact with hypoglycosylated IgG. ACPA are hypoglycosylated as compared with total IgG; thus, one might infer that RF can enhance the pathological effects of ACPA through preferential binding to ACPA and potentiation of the subsequent immune response.

Immunological consequences of ACPA deposition

To be effective, antibodies must, in general, recruit immune effector mechanisms mediated by activation of the complement system or Fc receptor-positive cells. The complement system can be activated via three pathways: the classical pathway, the lectin pathway and the alternative pathway. Each pathway is initiated by a specific recognition molecule. The classical pathway is initiated by complement C1q, the lectin pathway by mannose-binding lectin or ficolins, and the alternative pathway initiated through the spontaneous low level activation of C3. Initiation of complement activation via each of these pathways involves the formation of C3 convertase complexes (composed of different subunits in each pathway), which cleave complement C3 to produce biologically active complement fragments. These fragments attract and activate immune cells through their com-
plement receptors. Intriguingly, ACPA can activate human complement not only via the classical pathway, which is known to be activated by antibodies present in immune complexes, but also via the alternative pathway. These observations are reminiscent of findings from animal models of arthritis, which show that the alternative pathway of complement activation is crucially involved in autoantibody-mediated arthritis.

Besides activating complement, ACPA are also readily capable of triggering immune cell responses via Fc receptors (FcR). For example, immune complexes containing ACPA and citrullinated fibrinogen have been shown to trigger TNF secretion through engagement of FcγR on macrophages. Such findings should be interpreted in the perspective that many immune complexed model antigens, such as ovalbumin, elicit similar effects. Thus these data do not necessarily indicate the involvement of ACPA in disease pathogenesis. Nevertheless they are of relevance to understanding the pathogenic role of ACPA, as they show that these autoantibodies do have the potency to recruit powerful immune effector mechanisms.

This notion is further supported by observations made in animal models showing that ACPA, reactive with several citrullinated antigens are able to initiate and enhance arthritis. Findings made in these models also indicate that ACPA recognize citrullinated proteins in a hapten-like manner (that is, ACPA binds to citrulline as if it were a small molecule. This molecule can be recognized in the context of different protein backbones. Such a protein backbone is called a carrier which may be one that does not elicit an immune response by itself.). Thus, ACPA and anti-CCP2 antibodies crossreact with a variety of citrullinated proteins that bear little sequence homology; this crossreactivity, however, is not absolute, as indicated by studies using specific citrullinated peptides, some of which did not bind ACPA.

Altogether, these data provide fertile soil to fuel the hypothesis that ACPA play an important part in disease pathogenesis, although further evidence is required to substantiate this putative role in RA. In the following sections, we discuss characteristics of the ACPA response, and summarize emerging evidence for how these antibodies are related to clinical course and treatment outcomes in RA.

**ACPA characteristics**

The B-cell lineage generates antibody-secreting plasma cells and memory B cells, which have enhanced capability to respond to a specific initiating antigen by producing antibodies. The most important function of antibodies is protection of the host against invading pathogens, primarily through neutralization of molecules essential to the lifecycle of the pathogen and/or the recruitment of powerful immune effector mechanisms capable of killing pathogens or pathogen-infected cells.

To be properly effective, an antibody response needs to develop through avidity
maturation and isotype switching stages. T cells are intimately involved in these processes as they provide the helper activity essential for the maturation of most B cell responses (Figure 3). Similarly, diversification of the antibody response, through recognition of more epitopes, is thought to contribute to its efficacy.

**Figure 3. ACPA production by B cells can be stimulated by autoreactive T cells.**

Dendritic cells present peptides to naive T cells in complex with HLA class II molecules, activating the T cells and leading to TH cell-mediated stimulation of B cells. In the context of APCA production, it has been hypothesized that B cells can recognize citrullinated peptide complexes, for example on apoptotic cells. B cells can internalize these complexes, process them and present peptides from them in complex with HLA molecules encoded by SE alleles (these peptides may or may not be citrullinated), to T cells. When T cells recognize those peptides they can provide help to the B cells, resulting in the production of ACPA. Antibodies to different epitopes (including citrullinated epitopes) of the internalized peptide complex can be produced, leading to the production of ACPA. Therefore, T cell help stimulates the production and maturation of ACPA. Abbreviations: ACPA, anti-citrullinated protein antibodies; SE, shared epitope; TH cell, T helper cell.

**Fine specificity and epitope spreading**

ACPA can recognize a variety of citrullinated antigens, including citrullinated fibrinogen\(^{51,52}\), citrullinated vimentin (which is also known as the Sa antigen)\(^{53}\), citrullinated type II collagen\(^{54}\), citrullinated α-enolase\(^{55}\) and many more citrullinated proteins. An increase or shift in the antigen recognition profile (a phenomenon known as epitope spreading) can have important pathophysiological consequences, as has been described in, for example, systemic lupus erythematosus\(^{56}\) and pemphigus. Autoantibodies such as anti-desmoglein antibodies present in patients with pemphigus vulgaris have been convincingly shown to mediate a pathogenic effect, through transfer into experimental animals.\(^{57}\) Furthermore, in pemphigus
(of which there are two major types: pemphigus foliaceus and pemphigus vulgaris,) reactivity against different desmoglein epitopes is associated with different outcomes. In patients with pemphigus foliaceus, autoantibodies to desmoglein-1 occur. These autoantibodies mediate blistering of the skin through loss of adhesion in the superficial epidermis, where desmoglein-1, but not desmoglein-3, is expressed. By contrast, in mucosal pemphigus vulgaris, the presence of anti-desmoglein-3 IgG antibodies causes blistering of the mucosae, where desmoglein-3 is expressed. Importantly, the example of pemphigus elegantly demonstrates that intramolecular epitope spreading might modulate remissions and relapses, as autoantibodies that recognize the EC5 domain of desmoglein-1 seem to be nonpathogenic, whereas those directed against the EC1 and EC2 domain of the molecule are associated with disease onset and active disease. Passive transfer experiments have demonstrated that anti-EC1 and anti-EC2 autoantibodies are pathogenic, whereas anti-EC5 autoantibodies are incapable of inducing blisters in mice. Pemphigus is, therefore, a prototype disease that indicates the relevance of epitope spreading in the transition from the preclinical to the clinical stage of autoimmune disease.

The ACPA immune response in RA starts several years prior to diagnosis of the disease, even before the onset of symptoms, but in a restricted manner with low antibody titers and limited peptide reactivity. ACPA titers and peptide-recognition profiles increase as the individual approaches disease onset (figure 4a). Likewise, in patients with arthralgia, the development of arthritis is predicted not only by the presence of ACPA, but also by their levels. Indeed, patients with arthralgia who have an extended ACPA repertoire are at higher risk of developing arthritis. Similarly, ACPA-positive patients with early arthritis that do not fulfill the American College of Rheumatology (ACR) classification criteria for RA are more likely to develop RA if their ACPA response is reactive to more citrullinated epitopes. These findings are consistent with the notion that a ‘broader’ ACPA recognition profile is associated with the transition towards (persistent) disease, and resemble the observations made in pemphigus with the exception that, thus far, no specific anti-citrullinated epitope or protein reactivity has been identified that would predict disease course in RA. Given the hapten-like recognition of citrullinated antigens and the high crossreactivity towards multiple citrullinated proteins of the ACPA response, this lack of fine specificity is perhaps not surprising.

Isotype profiles

Isotype switching is another event involved in enhancing the efficacy of (auto) antibodies, and leads to an increase in the diversity of antibody structure that enables the activation of more immune effector mechanisms. ACPA can be present in different forms, including IgG, IgA, IgM and IgE (figure 4b).
Multiple ACPA isotypes are present before the onset of RA. Likewise, the ACPA isotype distribution does not seem to significantly expand anymore during disease progression from undifferentiated arthritis (UA) to RA, indicating that most of the expansion of isotype usage by ACPA takes place before the onset of arthritis.

**Figure 4. Fine specificity and isotype profiles are important characteristics of an ACPA response.**

A. ACPA can recognize different citrullinated antigens, including for example citrullinated vimentin, citrullinated fibrinogen and citrullinated α-enolase. Although it is the citrulline moiety that binds to the autoantibody, the context of the amino acids surrounding the citrulline are important for recognition by ACPA with differing fine specificities. The ACPA recognition profile seems to be established in undifferentiated arthritis, with a broader profile being associated with subsequent progression to persistent disease. The peptides presented by the B cells may or may not be citrullinated.

B. Different antibody isotypes can activate the immune system via different pathways. For example, IgM, IgA and the different IgG subclasses activate the complement system to different extents. Although IgG is the most common isotype of ACPA in RA, the other isotypes also occur...
in some patients. Different isotypes recruit different effector functions, for example, of IgE-ACPA it has been hypothesized that it can activate FcεR1-positive cells, such as mast cells, adding participation of these cells to a subsequent inflammatory process. Furthermore, the presence of IgM ACPA indicates an ongoing immune response, with recruitment of new B cells into the ACPA response.

Abbreviations: ACPA, anti-citrullinated protein antibodies; RA, rheumatoid arthritis.

**Maturation of the response**

During a B-cell response against recall antigens, isotype switching and affinity maturation typically occur in germinal centers. Following somatic hypermutation, different B-cell clones will compete for antigens presented on follicular dendritic cells. B cells that express immunoglobulins of sufficiently high avidity will acquire the signals necessary for survival and proliferation. As a result, the total avidity of the immune response—defined as the overall binding strength of polyclonal antibodies to a multivalent antigen—increases, because low avidity B cells will not be stimulated and will eventually disappear from the population. The avidity maturation of antibody responses against recall antigens, mostly following vaccination, has been studied extensively, but autoantibody responses seem to behave differently. For example, the avidity of ACPA is significantly lower than the avidity of antibodies to the recall antigens tetanus toxoid and diphtheria toxoid, pointing to a different regulation of autoantibody responses as compared with recall antigens. In individual patients with RA, ACPA do not show avidity maturation during longitudinal follow up and even in patients who displayed extensive isotype switching, ACPA avidity was relatively low, indicating that these two maturation processes are uncoupled in the ACPA response.

**Glycosylation**

Although the specificity of antibodies is determined by the variable region, antibody-mediated effector functions are crucially dependent on the interactions of its constant region (Fc part) with the complement system as well as with the Fc receptors. These Fc-mediated effects are influenced by Fc-linked carbohydrate structures known as glycans. By glycosylation, a posttranslational modification process, different kind of glycan structures can be attached to the Fc-parts of the antibodies, resulting in several different glycoforms in human serum. Glycosylation of the Fc-tail of antibodies affects the recruitment of effector function, especially the binding to pro-inflammatory respectively anti-inflammatory Fc-receptors. 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 remission. Scherer et al investigated whether the glycan chains carried by ACPA differ from
the glycans carried by other antibodies in the sera of RA patients. \cite{38} A comparison of serum ACPA IgG1 to total serum IgG1 revealed that ACPA were associated with a characteristic glycan profile lacking sialic acid residues. ACPA from synovial fluid of RA patients were highly agalactosylated and due to the attachment of sialic acid to galactose, thereby also lacked sialic acid. Since Fc glycosylation directly affects the recruitment of Fc-mediated effector mechanisms, these data could contribute to the further understanding of the role of ACPA in disease pathogenesis of RA.

The data described above indicate that ACPA-producing B cells behave differently from ‘conventional’ B cells. In 2010, rituximab was shown in mice to specifically deplete B cells that produce autoantibodies, while sparing the ‘conventional’ plasma cells that produce protective antibodies. \cite{72} Treatments that target the crucial biological mechanisms underlying ‘conventional’ B-cell responses might, therefore, prove not to be as effective as anticipated, because ACPA-producing B cells might be following other biological routes. \cite{73}

**Figure 5. The maturation of the anti-citrullinated antibody response in the development of RA.** The maturation of the dragon is depicted as a metaphor for the development of the ACPA response. Breaking of tolerance against citrullinated antigens can occur in healthy individuals, represented by the hatching of a dragon egg. Although immature in terms of isotype profile and breadth of antigen recognition, this early ACPA response might still be harmful, and potentially contributes to the progression of preclinical arthritis to early RA. We hypothesize that maturation of the ACPA response takes place during the preclinical phases, such that by the time early arthritis emerges clinically, the isotype and antigen-recognition profiles of the ACPA response are established. Greater breadth of ACPA isotype usage and/or recognition profiles predispose an individual to develop the full disease, but the ACPA response does not mature any further as the disease becomes more severe. Abbreviations: ACPA, anti-citrullinated protein antibodies; RA, rheumatoid arthritis.

Together, the collection of data described above provides credible support for the notion that the ACPA response shifts from ‘infancy’ to ‘adulthood’ before transition to clinical disease (Figure 5). Whether the maturation of the ACPA response is a consequence or a cause of disease initiation is not known, but identifying the
master switches responsible for the expansion of the ACPA response might be instrumental for further elucidation of the disease pathogenesis.

ACPA and clinical features

ACPA-positive and ACPA-negative disease have been shown to be associated with different genetic and environmental risk factors, fueling the hypothesis that different pathophysiological mechanisms underlie these two separate disease subsets. For example, ACPA-negative RA associates with HLA-DR, whereas the HLA shared epitope (SE) alleles predispose to ACPA-positive disease. Likewise, the contribution of smoking to disease risk is mainly confined to the ACPA-positive HLA-SE-positive patient group. Stratifying patients with RA on the basis of ACPA status has resulted in the identification of more homogenous patient groups, with respect to both disease course and response to treatment.

ACPA and treatment outcomes

So what are the therapeutic implications of subgrouping patients with RA according to ACPA status? Logically, diseases with distinct pathogenesis might benefit from different treatment strategies. Methotrexate is the most prominent DMARD. A few years ago, we performed a double-blind placebo controlled randomized trial comparing two treatment strategies in patients with UA. Interestingly, the outcome of this study indicated that ACPA-positive patients with UA treated with methotrexate are less likely to progress to RA, and do so at a later time point as compared with a placebo control group. Unexpectedly, no effect of methotrexate therapy on progression to RA in the ACPA-negative group was observed. Interestingly, among patients with UA, those with low or intermediate ACPA levels respond better to methotrexate than patients with high ACPA levels. The data from this randomized trial not only indicate that the two ACPA subgroups respond differently to methotrexate treatment, but also that in patients with high ACPA levels methotrexate monotherapy might be insufficient. Indeed, the presence of ACPA and IgM RF together with elevated levels of C-reactive protein is predictive of more rapid radiographic progression in patients with RA. Patients with ACPA and IgM RF are also more likely to respond insufficiently to methotrexate monotherapy for recent-onset RA.

It is not only in regard to response to DMARDs that ACPA status seems to matter. In a trial published in 2011, 208 patients with RA refractory to therapy with TNF blockers were treated with rituximab. Rituximab is a monoclonal antibody directed towards the B-cell marker CD20, and has been shown to be an effective treatment in RA. In these patients, the presence of ACPA predicted a better EULAR response to rituximab at 24 weeks. Thus, rituximab might have a greater role in
ACPA-positive patients with RA than in ACPA-negative individuals. The mechanisms of rituximab efficacy, and of B-cell involvement in RA, are incompletely known; the basis for these differing outcomes remains to be elucidated.

**ACPA, remission and long-term monitoring**

The absence of ACPA and IgM RF are independent predictors of drug-free remission. As we have outlined, the course of ACPA-positive disease seems to be characterized by more persistent inflammation than its ACPA-negative counterpart. Together, these data indicate that treatment decisions in RA can be guided by ACPA status. Seroconversion is uncommon among ACPA-positive and ACPA negative patients; therefore, it does not seem to be useful to repeat ACPA measurements in daily practice. Thus, these data support the hypothesis that RA can be classified into two different disease subsets, and suggest that developing different classification criteria for ACPA-positive and ACPA-negative RA might help to optimize treatment strategies.

**ACPA and disease outcome**

The emerging relevance of ACPA status to treatment decisions is not only based on differential treatment efficacies, but is also supported by differences in disease outcome. Typically, 50–70% of the patients with RA are ACPA positive. Although ACPA-positive and ACPA-negative patients with RA show a very similar clinical presentation in the early phase of the disease, their subsequent disease course is different—extra-articular manifestations are clearly influenced by ACPA status. For example, ACPA positivity is associated with an increased risk of developing ischemic heart disease or lung pathology. Likewise, ACPA-positive patients have more destructive disease than ACPA negative patients; ACPA-positive patients develop erosions earlier and more abundantly than patients without ACPA. Owing to their more severe disease-course, ACPA-positive patients require a more aggressive treatment regimen than ACPA-negative patients. Indeed, in the BeSt study ACPA-positive patients initially treated with DMARD monotherapy displayed greater radiographic joint destruction after 2 years than ACPA-negative patients. In patients initially treated with combination therapy, by contrast, no difference with respect to joint destruction was observed between ACPA-positive and ACPA-negative patients. These observations suggest that effective treatment with combination therapy, together with steering treatment according to disease activity, can prevent radiographic progression, even in patients with risk factors for severe damage, such as ACPA-positive patients.

The ACPA isotype profile has been associated with a higher risk of radiographic progression, leading to a 1.4-fold increase in risk per isotype used in the ACPA response, illustrating that an extended isotype usage is associated with a worse outcome.
Altogether, evidence is emerging that ACPA-positive and ACPA-negative RA represent two different disease entities with different outcomes, and, possibly, different responses to medication. The latter notion is especially important as it indicates that treatment regimes can be optimized by developing them according to ACPA status.

OUTLINE OF THIS THESIS

The general aim of this thesis was to elucidate the immunological properties of ACPA and to investigate the association between ACPA characteristics and the disease course of RA.

ACPA display high association with RA and are implicated in its pathogenesis. The presence of ACPAs is known to precede the onset of RA. North American natives have previously been reported to have a younger age at disease onset and an increased prevalence and severity of RA. Genetic studies have also revealed a higher prevalence of HLA DRB1 SE alleles in North American natives and increased frequency of RF positivity in patients with RA in several North American native populations. In order to identify the features of ACPA that could confer its pathogenicity, we extensively characterized this antibody response in a unique North American native population of patients with RA and their unaffected relatives in chapter 2.

Furthermore, the synovium is the primary site of pathology in RA, and ACPA are readily detectable in the synovial fluid of patients with RA. The transition from asymptomatic autoimmunity to clinically detectable synovitis is not well understood. Chapter 3 describes the serological and synovial features of a young woman from a multi-case RA family from a North American Native population who initially had asymptomatic autoimmunity, then subsequently developed clinical features suggestive of early RA.

HLA SE alleles are known to be associated with RA susceptibility, specifically susceptibility to ACPA-positive RA. Furthermore, smoking is known to predispose to the development of ACPA-positive RA. The combined effect of HLA SE alleles and smoking are known to exceed the sum of their single effects: a phenomenon known as biological interaction. Recent data suggest that a gene–environment interaction between smoking and the HLA shared epitope alleles plays a role in shaping the autoimmune reaction towards a specific citrullinated antigen. More recently, however, our group has reported a similar interaction for 2 peptides derived from 2 other citrullinated proteins, citrullinated vimentin and citrullinated fibrinogen. These latter data indicate that the observed interaction might not be confined to an autoimmune response against a specific citrullinated antigen. Rather, these findings indicate that these interaction effects extend to several citrullinated autoantigens, which might even be explained by a gener-
al predisposition to ACPA development. In chapter 4, we aimed to analyze in greater detail the extent of the interaction between HLA shared epitope alleles and tobacco exposure on the antigen-recognition profile of ACPA, especially in ACPA-positive patients in order to exclude the possibility that the association is caused by the presence of ACPA rather than by the presence of an autoimmune reaction to specific citrullinated epitopes.

Another citrullinated protein in inflamed synovial tissue identified previously is fibronectin (FN). FN, one of the most abundant proteins present in the inflamed joint, is a glycoprotein, which can be a component of the extracellular matrix (insoluble form) or present in body fluids (soluble form). FN is involved in a variety of processes, such as wound healing, haemostasis, thrombosis and embryogenesis. In chapter 5 we characterized the citrullination of fibronectin in the joints of RA patients and studied the prevalence, epitope specificity and HLA association of autoantibodies against citrullinated fibronectin in RA.

HLA SE alleles are not only associated with the presence of ACPA, but also with joint destruction and the ACPA fine specificity repertoire. A large variation in joint destruction is seen within the ACPA-positive patient population. It is conceivable that certain citrullinated antigens are more potent than others in activating T cells in the context of HLA SE alleles. HLA SE alleles were found to associate with antibodies targeting peptides from citrullinated vimentin, but not with the presence of antibodies recognizing citrullinated fibrinogen. This differential modulation of the ACPA response by SE-alleles, and the fact that disease phenotypes vary greatly among ACPA-positive patients, has raised the question whether certain ACPA fine-specificities might associate with a more severe disease phenotype. If so, designing assays that test for these specificities would be of prognostic value and could influence treatment decisions in the clinic. Therefore, in chapter 6 we investigated whether ACPA fine-specificities, which are formed under the influence of SE-alleles, associate with the extent of radiographic joint damage.

Furthermore, it is conceivable that a certain ACPA recognition profile is associated with the emergence of certain clinical features and possibly pathogenicity in RA, as has been shown in other autoimmune diseases. For example, in pemphigus the reactivity against different desmoglein epitopes is associated with different outcomes. In chapter 7 we determined the association between the ACPA fine specificity and phenotypic characteristics within ACPA-positive RA and investigated whether specific subsets of RA patients can be distinguished on the basis of their epitope recognition profile.

Very early therapy in RA with disease-modifying antirheumatic drugs is associated with lower levels of joint destruction and a higher chance on achieving remission. Having symptoms for >12 weeks at treatment initiation is a strong and independent risk factors for a persistent disease course. These observations have led to the hypothesis that the ‘window of opportunity’ exist early in dis-
This hypothesis presumes that underlying disease processes are not fully matured in the very early stage of arthritis, making modulation more successful. As ACPA precede arthritis development and are associated with a severe disease course, we hypothesized that the ACPA response broadens within the very early phase of RA and in doing so limits the 'window of opportunity'. Therefore, it was examined in chapter 8 whether patients who were assessed within 12 weeks of symptom onset have a less broadened ACPA response than patients with longer symptom duration.

The ACPA response likely represents a T-cell-dependent B-cell response, given the protein nature of the antigen recognized and the strong association with the HLA SE alleles. The evolution of such a response is typically characterized by a first wave of IgM antibodies after the first antigen contact, quickly followed by the presence of IgG. After repeated antigen exposure, the IgG responses are further boosted while the IgM peak declines. Therefore, it is conceivable that the presence of IgM ACPA suggests that activation of recently recruited naïve B cells recognize citrullinated antigens because the half-life of circulating IgM is short. In chapter 9 we determined whether there is a difference in the fine specificity of IgG and IgM ACPA.

Previously it has been shown that the levels of anti-CCP2-antibodies are higher in synovial-fluid than serum. However, very limited information on absolute levels of ACPA in either synovial-fluid or serum is present as the levels are generally expressed as arbitrary units. Nonetheless, it is interesting to obtain information on the absolute concentration of ACPA as this would allow the comparison of the ACPA-response to other antibody responses in quantitative terms. In order to quantitate ACPA-levels it is required to isolate ACPA. In chapter 10 we present data on the estimation of the minimal ACPA quantities in serum and synovial-fluid.

Recently, a novel family of autoantibodies in RA patients was described: anti-carbamylated protein (Anti-CarP) antibodies, which target carbamylated (homocitrulline-containing) epitopes. Since citrulline and homocitrulline have a similar structure, in chapter 11, we wished to determine to what extent human autoantibodies can differentiate between them. Unlike human antibodies, the anti-modified citrulline (AMC) antibody developed by Dr Senshu is able to recognise citrullinated epitopes irrespective of the neighbouring amino acids. Therefore, we also aimed in to verify whether the AMC assay could distinguish between these two amino acids.

Finally, chapter 12, provides a summary of the results and a discussion of the implication of the studies described in this thesis.
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