Anti-CCP antibodies: the past, the present and the future

Walther J. van Venrooij, Joyce J. B. C. van Beers and Ger J. M. Pruijn

Abstract | Rheumatoid arthritis (RA) is an autoimmune disease characterized by autoantibodies against citrullinated antigens. The importance of citrulline for the epitopes bound by these autoantibodies, referred to as ACPA (anti-citrullinated peptide/protein antibodies), was first described in 1998. In addition to citrullinated proteins, cyclic citrullinated peptides (CCP) can also be used as test substrates for detecting ACPA. The standard test for these antibodies is the second-generation CCP (CCP2) test, which is one of the best in terms of sensitivity and specificity. The generation of ACPA is an early event in the disease course, and is dependent on the presence of certain MHC class II alleles. ACPA in the inflamed synovium have been shown to associate with citrullinated antigens to form immune complexes, resulting in progression of the inflammatory process. The involvement of ACPA in the chronicity of RA is probably the reason why ACPA-positive patients have a more erosive disease course than ACPA-negative patients. The presence of ACPA has been included in the 2010 RA classification criteria. Thus, it is important to further standardize ACPA testing, for example by including an internal serum standard, which may lead to a better distinction between low and high ACPA levels.

van Venrooij, W. J. *et al. Nat. Rev. Rheumatol.* **7**, 391–398 (2011); published online 7 June 2011; doi:10.1038/nrrheum.2011.76

Introduction

Rheumatoid arthritis (RA) is a common autoimmune disease characterized by chronic inflammation of the synovial joints. In most cases, this chronic inflammation will induce the formation of pannus tissue, ultimately leading to joint destruction. In 1998, the interesting finding that patients with RA produce antibodies against peptides and proteins containing citrulline, a modified form of the amino acid arginine, was first published.[1] Based on this finding, a commercial test was developed using synthetic cyclic citrullinated peptide (CCP) as an artificial antigen.[2] Subsequently, a large number of publications have demonstrated that the use of this test enables early diagnosis and treatment of RA.[2–8]

Testing for these antibodies has been included as a new serologic criterion in the recently published 2010 RA classification criteria.[9–11] In this Review, we discuss the past, present and future use of this biomarker, not only for the management of RA but also for predicting the course of this disease.

The past

In 1964, Nienhuis and Mandema[12] reported that antibodies in sera from patients with RA reacted in a very specific way with keratohyalin granules present in buccal mucosa cells (Figure 1). A serological test based on this finding became known as the anti-perinuclear-factor (APF) test.

The biochemical identity of the antigen(s) in the keratohyalin granules remained unknown for many years, although in 1985 one component of these granules was identified as filaggrin or its precursor profilaggrin.[13] Several years later, Hoet and colleagues[14] demonstrated that the perinuclear factor exactly co-localized with (pro)filaggrin, but attempts to show that the antigen was identical to this protein failed. However, they noted that the antigen was only present in the fully differentiated squamous epithelial cell layers. In a follow-up study, the same investigators found that the antigen was not present in keratohyalin granules of cultured buccal mucosa cells, despite the presence of (pro)filaggrin in these granules.[15] A few years later, a group led by Guy Serre convincingly showed that filaggrin in differentiated skin tissue was identical to the perinuclear factor.[16,17] Given the apparently discrepant results between these reports, an explanation that could be forwarded was that the mature filaggrin in fully differentiated epithelium contains a partially different chemical structure from the profilaggrin in cultured buccal mucosa cells. This led to the hypothesis that the (pro)filaggrin protein becomes modified during differentiation of epidermal cells, and that this modification in the mature filaggrin protein is essential for its antigenicity. The most prominent modification of filaggrin that was known at that time was the presence of citrulline, a deiminated product of the amino acid arginine. Filaggrin in fully differentiated buccal mucosa cells (largely dead cells) is citrullinated, in contrast to profilaggrin in cultured, living cells (Box 1).

These assumptions turned out to be correct, and it was indeed shown that citrulline is an essential constituent of the antigenic determinants recognized by RA-specific autoantibodies.[1] This finding was subsequently confirmed

Competing interests
The authors declare no competing interests.

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Key points

- Anti-citrullinated peptide/protein antibodies (ACPA) are present in early disease, and are highly specific for rheumatoid arthritis (RA)
- Data from the literature show that the second-generation anti-cyclic citrullinated peptide antibody (CCP2) test is one of the best tools for detecting ACPA
- The CCP2 test enables the clinician to distinguish two subclasses of patients with early RA (ACPA-positive and ACPA-negative), each with their own genetic background and future disease course
- ACPA have recently been added to the RA classification criteria jointly developed by the American College of Rheumatology and the European League Against Rheumatism

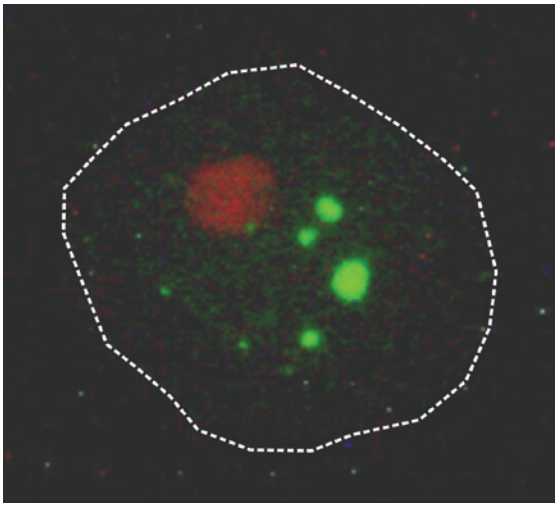

Figure 1 | Typical immunofluorescence staining pattern of a buccal mucosa cell by APF-positive RA serum. The borders of the buccal mucosa cell are marked by a dashed line. The nucleus is counter-stained with ethidium bromide (red). The keratohyalin granules are stained by patient antibodies via a secondary fluorescently labeled anti-human-IgG antibody (green). Note that superficial buccal mucosa cells are actually dead cells that can easily be scraped off from the inside of the human cheek. In such fully differentiated cells, the filaggrin protein is citrullinated. Abbreviations: APF, anti-perinuclear factor; RA, rheumatoid arthritis.

by Serre's group.[18] The first tests for the presence of these antibodies employed either linear citrullinated peptides or citrullinated filaggrin as an antigen, and, although they showed excellent specificity, the sensitivities obtained were, in most cases, relatively low.[19] To increase the sensitivity of the citrulline-containing peptide ELISA (enzyme-linked immunosorbent assay) used by Schellekens and colleagues,[1] the peptides were made cyclic (that is, modified to adopt a structure in which the citrullinated epitope is more efficiently recognized by the patient's antibodies).[2] Using this approach of a single cyclic citrullinated peptide (CCP), antibodies could be detected in 68% of RA sera with a specificity of 98%.

The first-generation CCP ELISA test (the CCP1 test) used a filaggrin-derived cyclic peptide as the antigenic substrate.[2] As filaggrin is not present in the inflamed joint, we hypothesized that better antigenic peptides, possibly derived from citrullinated synovial proteins, could be found. About 12 million peptides from dedicated synthetic peptide libraries were subsequently screened with RA sera, and the best citrullinated peptides were incorporated into a new format, which was referred to in the literature as the second-generation CCP test (CCP2 test). This test first became commercially available in 2002 and has not been changed since then, although CCP2 ELISA tests are nowadays supplied by many companies, and CCP2 tests have been developed for several automated analyzers as well. According to the literature, the CCP2 test is still recognized as the gold standard of testing for anti-citrullinated peptide/protein antibodies (ACPA).[7,20]

The present

The immune response to citrullinated proteins

Citrullination is a normal physiological process that occurs inside many dying cells of the body (Box 1, Figure 2). It is, therefore, important to realize that the immune system normally does not encounter citrullinated proteins. Soon after the change in phenotype of the dying cell, it is ingested by macrophages and other cells active in the specific clearance of apoptotic cells. When the clearance system is inefficient or of inadequate capacity, such as when massive cell death occurs, pepdidylarginine deiminase (PAD) enzymes and citrullinated proteins can leak from the necrotizing cell and 'meet' the immune system.

The released PAD enzymes will citrullinate many extracellular proteins containing arginine, thus creating a large pool of citrullinated antigens.[21] During inflammation, when many cells die by apoptosis or necrosis, it is possible to detect citrullinated proteins at the site of inflammation, both in animal models of inflammation and in the inflamed synovial tissues of patients with or without RA.[22] However, the presence of citrullinated proteins does not necessarily lead to the generation of ACPA, as the production of such antibodies is thought to be strongly dependent on the genetic background of the patient, as discussed below.

Generation of ACPA is MHC dependent

For over four decades it has been known that certain MHC class II alleles are a major genetic risk factor for RA. Indeed, numerous studies have shown the association between HLA-DRB1 shared epitope (SE) alleles and RA.[23] In a seminal paper, Hill *et al.*[24] demonstrated that the conversion of arginine to citrulline at the peptide side-chain position that interacts with the SE significantly increases peptide–MHC affinity and leads to the activation of $CD4^+$ T cells in DR4-IE transgenic mice. A few years later, these authors showed that citrullinated fibrinogen is able to induce arthritis in these transgenic mice. T-cell epitope scanning and antibody microarray analysis identified a unique pattern of citrulline-specific reactivity that was not found in DR4-IE transgenic mice immunized with unmodified fibrinogen or in the corresponding wild-type C57BL/6 mice immunized with citrullinated fibrinogen.[25] Conversion of arginine into citrulline generates 'altered self' peptides that can

be bound and presented by DRB1*1001, one of several SE alleles[26] that is also strongly associated with RA and ACPA. James *et al.*[27] showed that the accommodation of the citrulline moiety can occur in multiple pockets (positions 4, 7 and 9) of DRB1*1001. Using a new classification of the SE, Gyetvai *et al.*[28] showed that, in particular, the S2 and S3P alleles (both associated with increased risk of RA) predisposed individuals to the production of anti-CCP and anti-MCV (mutated citrullinated vimentin) antibodies. Antibodies against citrullinated fibrinogen had a different association pattern, and correlated best with the S1 allele.

Thus, from these and many other studies, it can be concluded that the SE significantly increases the risk of developing ACPA.[29–31] The production of ACPA can ultimately lead to the formation of immune complexes and continuation of inflammation, with the final outcome of chronic joint inflammation, which we refer to as RA.[32,33]

The CCP2 test

The CCP2 test has been used extensively by laboratories all around the world: the accumulated specificity and sensitivity figures from 164 studies published over the past 8 years are presented in Table 1. Owing to the widespread use of testing for anti-CCP antibodies and other ACPA in routine settings, this serologic parameter was recently included in the new 2010 RA classification criteria composed by a joint working group of the ACR (American College of Rheumatology) and EULAR (European League Against Rheumatism). In this new classification system, parameters were selected that are detectable at early stages of disease rather than those that define the disease by its late-stage features, as was the case in the 1987 ACR criteria.[9] As it is likely that these new classification criteria will be rapidly adopted in daily practice,[11] it is important to know which ACPA test is best suited for the clinician.

As several new ACPA tests have become commercially available in recent years (MCV, CCP3 and others), a detailed analysis of the literature was performed to establish how these tests behave in comparison with the CCP2 test. Indeed, in some publications, ACPA tests have been described as being somewhat more sensitive (for example, the anti-MCV test)[34–36] than the CCP2 test; however, in such cases the anti-MCV test almost always shows a lower specificity as well.[37,38] It is well known that diagnostic tests should be compared only under stratified conditions (for example, the comparison of sensitivities at a fixed clinical specificity), so that correct positive and negative predictive values, as well as positive and negative likelihood ratios, can be calculated. In several published articles, such a comparison between different commercial ACPA tests showed that, at a stratified specificity, none of the presently available ACPA tests has a higher sensitivity than the CCP2 test.[37–44]

It is also interesting to note that high levels of rheumatoid factor (RF)—although still included in the 2010 RA criteria—seem to have limited value when compared to ACPA positivity.[45] An additional advantage of ACPA testing is the recent finding that it can distinguish between two distinct groups of RA patients, namely those who are ACPA-positive and those who are ACPA-negative.

Box 1 | Citrullination

Citrullination of proteins is a process that occurs almost exclusively in dying cells, the reason being that the enzymes that catalyze the conversion of peptidylarginine into peptidylcitrulline are only active when Ca^{2+} concentrations are $\geq 10^{-5}$ mol/l. Normal cellular Ca^{2+} concentrations are about 100 times lower. During cell death (apoptosis or necrosis) the cell membrane becomes leaky, allowing an unlimited influx of extracellular Ca^{2+} ions. As a consequence, the peptidylarginine deiminase enzymes become activated and start the modification of peptidylarginine into peptidylcitrulline. The effects of citrullination can be dramatic for the protein: due to the loss of positive charge, the citrullinated protein loses intramolecular and intermolecular interactions, unfolds, and subsequently is rapidly degraded by cellular proteases. In this way, many cellular proteins will lose their activity and many molecular machines will be destroyed. One of the first cellular proteins to become citrullinated is vimentin. This cytoskeletal protein is important for the structural integrity of the cell, and loss of its function will change the phenotype of the dying cell dramatically. Other intracellular proteins that become rapidly citrullinated are the nuclear histones, which are basic proteins essential for chromatin structure. Indeed, the cell-death process is profoundly accelerated by the citrullination of intracellular proteins.

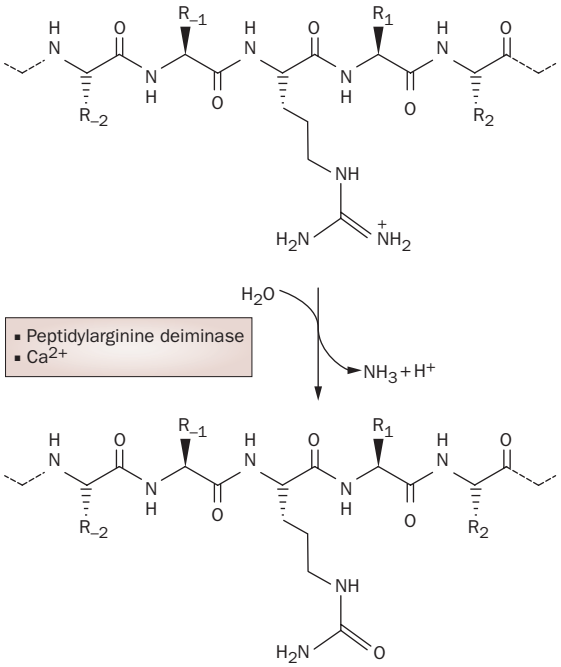

Figure 2 | Citrullination of proteins. Citrullination is the post-translational conversion of peptidylarginine into peptidylcitrulline (the guanidine group of the arginine side chain is converted into an ureido group), and is catalyzed by Ca^{2+}-dependent peptidylarginine deiminase enzymes. R_{-2} through R_2 refer to the side chains of the amino acids flanking the citrullinated residue.[89]

Diversity of the ACPA pool

In our RA cycle hypothesis[32] (Figure 3), we postulated that active PAD enzymes (and citrullinated cellular proteins like vimentin and histones) are released when the apoptotic clearance mechanism fails to remove the dying cells in time. One could speculate that the released citrullinated proteins function as the first citrullinated antigens

Table 1 | Specificity and sensitivity of the CCP2 test for RA

Patient group	Patients/ controls (*n*)*	Positive CCP2 test (*n*)	Sensitivity (%)	Specificity (%)
RA total	18,061	12,953	71.7	NA
Early RA	4,589	2,827	61.6	NA
Established RA	13,472	10,126	75.2	NA
Controls	20,908	1,010	4.8	95.2
Non-RA‡	15,971	960	6.0	94.0
Healthy	4,937	50	1.0	99.0

*Accumulated data from 164 studies published between 2002 and 2010. ‡Patients with a rheumatic or inflammatory disease other than RA. Abbreviations: CCP2 test, second-generation anti-cyclic-citrullinated-peptide antibody test; NA, not applicable; RA, rheumatoid arthritis.

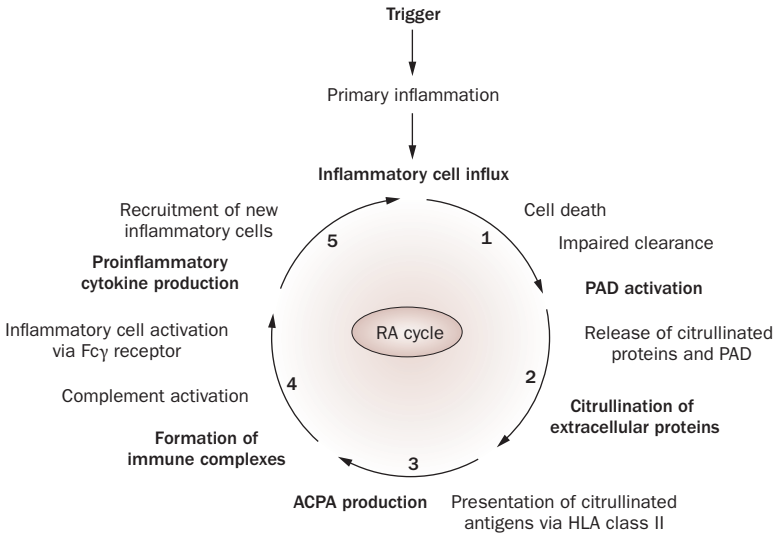

Figure 3 | The hypothesized role of citrullination in RA. Inflammation of the joint leads to infiltration of immune cells (step 1), which contain PAD enzymes. After PAD activation (step 2) due to a rise in intracellular calcium concentration during cell death (step 1), target antigens can become citrullinated. The apoptotic cell will normally be removed by neighboring phagocytic cells. However, when the number of dying cells is too high or when there is a defect in the clearance of apoptotic cell remnants, the cells can become necrotic and release their contents into the extracellular space. PAD enzymes will then also citrullinate extracellular proteins (step 2). In a small percentage of individuals, citrullinated proteins are exposed to the immune system, eliciting an immune response and the formation of ACPA (step 3). This will ultimately result in immune complex formation (step 4), followed by upregulation of proinflammatory cytokines (step 5), which are regarded as the driving force of the chronic inflammation that is typical of RA. Abbreviations: ACPA, anti-citrullinated protein antibodies; PAD, peptidylarginine deiminase; RA, rheumatoid arthritis.

to be encountered by the immune system. The released and activated PAD enzymes will, in addition, citrullinate many other arginine-containing proteins in the synovium (such as collagen and fibrin/fibrinogen), leading to the exposure of a secondary array of citrullinated peptides to the immune system. The resulting diversification of the ACPA pool is an early step in the RA cycle, and definitely occurs before the disease is clinically recognized.

Recent studies seem to confirm most steps of this RA cycle. Jansen *et al.*[46] described the predictive value of ACPA in early arthritis. Moreover, van der Woude and colleagues[8] showed that epitope spreading of the ACPA response occurs before disease onset, with an increase in the number of citrullinated antigens recognized. Furthermore, they showed that this extensive recognition pattern of different citrullinated peptides in patients who present with undifferentiated arthritis is associated with rapid disease progression to RA in the first year of follow-up. The recognition of multiple citrullinated antigens is only observed in ACPA-positive RA, and is associated with *HLA-DRB1*04*.[47] Although cross-reactivity of ACPA with multiple citrullinated epitopes may occur, and could explain the recognition of multiple epitopes by a single antibody, the majority of ACPA seem to display distinct antigen-binding specificities.[48,49]

Also in line with our RA cycle model is the finding that levels of specific ACPAs are elevated in synovial fluid, suggesting local antibody production and/or retention of ACPA at the site of inflammation.[48] PAD2, which is present in macrophages, and PAD4, which is found in leukocytes, are both present at sites of inflammation. As it is not unreasonable to assume that a PAD enzyme can auto-citrullinate itself,[50] the presence of citrullinated PAD enzymes in the inflamed synovium may also lead to the generation of anti-PAD antibodies. Indeed, serum IgG autoantibodies against PAD4 seem to be associated with anti-CCP antibody positivity, and have been detected in about 20–40% of patients with RA[51,52] and in the preclinical phase of RA in a subset of patients.[53]

Most ACPAs are IgG molecules, although IgA, IgM and IgE antibodies have also been described.[54–56] An interesting development is the recent finding that the presence of certain ACPA isotypes at baseline seems to indicate an increased risk of future radiographic damage.[54] The presence and constitution of the ACPA response might, therefore, be relevant to the RA disease course.[8]

The data in Table 1 show that anti-CCP2 reactivity is more frequently found in non-RA disease controls (generally patients suffering from other rheumatic and inflammatory diseases) than in healthy controls. From several studies it became clear that a positive reaction of non-RA sera (for example, that from patients with systemic lupus erythematosus, autoimmune hepatitis or tuberculosis) in an ACPA test is often due to the presence of antibodies that recognize the non-citrullinated target molecule.[57–60] It is advisable, therefore, to include a non-citrullinated control antigen in the test when these sera are being analyzed. Such control plates are available for the CCP2 test.

ACPA predict development of more-erosive RA

In 2003, it was first reported that anti-CCP antibodies can be present years before the appearance of the first clinical symptoms of arthritis.[4] This finding was subsequently confirmed by two other groups.[61,62]

As already noted above, it has recently become clear that RA patients can be classified into two major subsets: ACPA-positive and ACPA-negative.[63–65] Although these two groups of patients show a very similar clinical presentation in the early phase of the disease,[23,66] the picture changes considerably as the disease progresses, as ACPA-positive status is strongly associated with the development of more erosive disease. In this regard, it is important to note that environmental factors, such as

cigarette smoking, increase the risk of developing ACPA, and that ACPA positivity increases the risk of developing ischemic heart disease.[67,68] Furthermore, it was reported that treatment with methotrexate induced remission in some ACPA-positive patients with early arthritis but had no such effect in ACPA-negative patients.[69] Another issue is the question of whether seroconversion (ACPA-negative patients becoming ACPA-positive) can take place. To our knowledge, there are no published data regarding such conversions, and our own experience is that such conversions may occur only very early in disease, which will at least in part be due to significantly increased anti-CCP antibody titers. In this respect, it is important to note that the prevalence of anti-CCP antibodies in patients with early RA is generally lower than that in patients with established RA (Table 1). When a patient is ACPA-positive, he or she generally remains so. In most cases a slow increase in anti-CCP antibody titer may be observed (W. J. van Venrooij, unpublished data).[4]

Pathological role of ACPA in RA development

As mentioned above, genetic and environmental factors, including cigarette smoking, have an important role in the pathogenesis of RA,[23,70–72] and we have postulated the same for citrullination.[19,32,73] Several studies have already indicated that ACPA antedate clinical disease development.[4,61,62] Furthermore, in patients with arthralgia, the presence of ACPA or anti-CCP antibodies, but not RF or SE, predicts subsequent arthritis development,[74] suggesting that citrullination may be involved in the development of ACPA-positive RA.[32] Indeed, ACPA positivity and small-joint arthritis are consistent predictors of chronic arthritis in patients with very early arthritis.[75]

Some studies performed in experimental animal models have shown that ACPA can not only induce but also enhance arthritis. Transfer of a monoclonal antibody against citrullinated fibrinogen enhanced mild collagen-induced arthritis in a mouse model. By contrast, no arthritis was seen in naive animals with no joint lesions.[76] Additionally, inflammatory arthritis can be induced in experimental mouse models by either citrullinated collagen type II or citrullinated fibrinogen.[25,77,78] Mice with fibrinogen-induced arthritis have also been shown to produce elevated levels of RF, antibodies against native and citrullinated fibrinogen, immune complexes, and anti-CCP antibodies.[78]

In an *in vitro* model, Clavel *et al.*[79] showed that ACPA-containing immune complexes induced tumor necrosis factor secretion by human macrophages via engagement of FcγRIIa at the surface of these cells. Synovitis in ACPA-positive RA patients is associated with an increased number of infiltrating lymphocytes and a higher rate of local joint destruction than that in ACPA-negative patients.[80] ACPA or anti-CCP antibodies activate the complement system *in vitro* via the classical and alternative pathways—a strong indication of their pathophysiologic involvement.[81] These findings were confirmed and extended by Sokolove and colleagues,[82] who showed that citrullination boosts the local inflammatory response at sites of damage or inflammation, indicating the pathogenic specificity of an ACPA target. Although this issue is far from settled, the studies mentioned above are consistent with the notion that the pathogenesis of RA is mediated by citrullinated antigens and autoantibodies directed against them.

The future

With the inclusion of ACPA and/or anti-CCP antibody testing in the ACR–EULAR 2010 RA classification criteria,[9] citrullination and its role in the pathophysiology of RA will remain an important topic for further studies. These future studies may be focused in the following ways: the test, standardization of the test, and treatment.

The test

Although the CCP2 test could remain the gold standard of ACPA testing for the next few years, other commercial tests like the MCV and CCP3 tests might be used to detect further smaller subgroups in the heterogeneous population of patients with early RA. Also, new ACPA tests based on additional citrullinated autoantigenic proteins, such as α-enolase, might be developed to differentiate between clinically distinct RA patient subgroups.[83] A promising new approach is to look more into ACPA isotype profiles and changes therein after treatment. In this respect, it is interesting to note that low and intermediate pretreatment ACPA levels seem to be associated with a more favorable response to methotrexate treatment in recent-onset, ACPA-positive RA, whereas high levels are associated with an insufficient response.[84]

Test standardization

In the context of the new ACR–EULAR criteria, it is becoming increasingly important to standardize the available ACPA tests. Currently, CCP2 test kits are made by several companies. Although they all use the same antigens, the CCP2 kits may differ occasionally in sensitivity, even at stratified specificity (for example, the INOVA Diagnostic [San Diego, CA] CCP2 test shows significantly lower sensitivity at stratified specificity than other commercial CCP2 tests),[37] and have different cut-off values. We believe that these discrepancies can be resolved, at least in part, by including an internal serum standard with the test, which is available via the Centers for Disease Control and Prevention in Atlanta, USA. This may also lead to a better distinction between low and high ACPA levels, which is important for the 2010 RA classification criteria.[9]

Treatment

All the steps leading to the chronic inflammatory state that we refer to as RA, and first postulated by us in 2004[19,73] and in later reviews,[21,32] have now been experimentally proven. Thus, we can assume that the successive steps in the RA cycle (Figure 3) probably depict the development of RA.

Each step, though, has its own characteristics and variations. For example, environmental factors, such as smoking and hormone levels, might influence the first three steps, and the possible occurrence of bacterial PAD

enzymes[85,86] might be involved in an alternative version of the first two steps. Besides environmental factors, genetic factors, such as the HLA-DRB1 SE alleles, might be associated with step 3. By contrast, it has been suggested that a protective HLA-DRB1 allele (namely *HLA-DRB1*1301*) conveys protection in ACPA-positive, SE-positive RA patients.[26]

The RA cycle might also give us clues about the development of new therapeutic strategies. Most current treatments (such as biologic agents and methotrexate) are directed at step 5 (that is, to achieve decreased inflammation and cytokine production). Other approaches have targeted step 3 by decreasing antibody production via B-cell depletion therapy.[87,88] However, despite such treatments, the production of citrullinated antigens and ACPA continues, as does the production of immune complexes that stimulate inflammation. An alternative, and possibly very effective treatment, therefore, would be to block extracellular PAD activity (step 2) by applying a specific PAD2/PAD4 inhibitor. Such a treatment would inhibit the production of citrullinated antigens and, consequently, the production of ACPA and immune complexes.

Although ACPA were detected more than 40 years ago, and the recognition of their diagnostic value has steadily increased since, the development of more specific and less laborious tests for the detection of ACPA in the past decade has only recently led to their inclusion in RA classification criteria. Ongoing and future research will lead to a better understanding of the role of ACPA in RA chronicity and, possibly, identification of new targets for treatment that are based on protein citrullination and the anti-citrullinated protein response. In view of the specificity of this response for RA, therapeutic strategies aimed at the citrulline-related steps of the RA cycle may be more specific and, therefore, arguably less prone to adverse effects than the currently applied therapies that target the more general factors of inflammation.

The fact that the presence or absence of ACPA can differentiate between two clinically distinct subgroups of patients with RA raises the question of whether a specific, non-citrulline-dependent immune response is associated with the ACPA-negative group. Future research should be aimed at the identification of other autoantibodies that might be specifically produced by this patient group. It is tempting to speculate that, also in ACPA-negative patients, the immune response is directed against neoepitopes that are generated in terminally differentiated or dying cells, and that there are also specific HLA alleles associated with the immune response to these epitopes, as seen in ACPA-positive patients. In this regard, the possibility that the ACPA-negative group represents a combination of several distinct subgroups, rather than a single homogeneous group, should also be acknowledged.

Conclusion

ACPA have emerged as one of the most important biomarkers in RA, and their detection has been greatly facilitated by the development of the CCP test. ACPA might have a key role in the pathophysiology of RA, as they are already present early in the disease course, are highly specific for the disease, can predict disease development, and can cause or enhance arthritis in experimental animal models. The use of different ACPA tests in parallel could enable the differentiation between distinct ACPA-positive subgroups. In view of the new ACR–EULAR criteria for the classification of RA, there is an increased need for standardization of the different tests used to measure ACPA levels in patients' sera. On the basis of accumulating evidence that the recognition of citrullinated antigens by ACPA contributes to the disease process in patients with ACPA-positive RA, it is tempting to speculate about new therapeutic strategies that are focused on interfering with the production of citrullinated antigens and/or ACPA, or on blocking their interaction. Taken together, these issues might lead to a better and more personalized treatment of patients with RA in the future.

Review criteria

We searched for original articles focusing on anti-citrullinated protein antibodies in MEDLINE and PubMed published between 2007 and 2011. The search terms we used were "CCP", "citrullin*", "ACPA" and "rheumatoid". All papers identified were English-language full text papers. We also searched the reference lists of identified articles for further papers.

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Acknowledgments
Our work was supported in part by the Dutch Arthritis Association and the Dutch Technology Foundation (STW).

Author contributions
All authors took part in researching data for the article. W. J. van Venrooij and G. J. M. Pruijn discussed the content. All authors contributed equally to writing the article and reviewing/editing the manuscript before submission.