Adaptive immunity in rheumatoid arthritis: anticitrulline and other antibodies in the pathogenesis of rheumatoid arthritis

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Purpose of review
To describe recent progress concerning rheumatoid arthritis (RA)-associated autoantibodies, in particular antibodies to citrullinated proteins antigens.

Recent findings
An increasingly diverse and RA-associated repertoire of antibodies has been defined over the last few years. These antibodies are preferentially, but not exclusively, reactive with posttranslationally modified antigens. Citrullinated antigens are the most common targets, but also other modifications including homocitrullination (carbamylation) are recognized. These antibodies display varying degrees of cross-reactivity, and both broadly cross-reactive and monoreactive antibodies are present. Progress, described in this review, has been made both concerning mechanisms behind the generation of these antibodies and concerning their effector functions.

Summary
Several different triggering mechanisms are involved in forming an antibody repertoire that evolves before the onset of clinical disease, and where antibodies with different specificities may interact directly or indirectly with target organs in causing different arthritis-associated symptoms. The increasing understanding of the role of adaptive and specific immunity in RA creates opportunities for a new generation of interventions.

Keywords
anticitrulline protein antibodies, cartilage-derived autoantigens, immunotherapy, posttranslational modifications

INTRODUCTION
Experience from animal experiments has taught us that there are many different routes to arthritis, where some involve adaptive immunity and others depend on aberrant regulation of innate immunity [1]. Aberrant immunity contributing to arthritis may have many different targets, involving ubiquitous antigens such as glucose phosphate isomerase (GPI) [2], as well as organ-specific antigens including a series of cartilage-derived proteins [3,4]. Notably, though, man-made genetic manipulations or immunization protocols are most often involved in the generation of these models, demonstrating that these animal experiments visualize potential pathways that may be involved in disease, but do not provide any information on whether these pathways are ever activated in humans. Important lessons from these models are, however, that specific immunity associated with arthritis may both induce disease and protect against disease, and that different specific immune reactions may cause joint inflammation as well as other arthritis-associated symptoms such as pain [5∗] or osteopenia [6∗∗].

A key message for this review is thus that there may be many routes to arthritis also in humans, and that we have to consider immunities directed toward several different antigens, organ-specific as well as ubiquitous. Furthermore, we have to acknowledge that observed arthritis-associated immunities in humans may be disease – inducing,
KEY POINTS

- ACPAs and other autoantibodies are associated with different subsets of RA. Antibodies with different specificities are associated with different genetic variants and with different environmental factors.
- ACPAs and other autoantibodies present in RA patients have different fine specificities and different degrees of cross-reactivity with various citrullinated and/or otherwise posttranslationally modified peptides/proteins.
- Different ACPAs and other RA-associated antibodies have different capacities to cause and/or contribute to different arthritis-associated symptoms, and specific mechanisms associated with a ‘second signal’ may be needed before ACPAs contribute to disease symptoms.
- ACPAs and other RA-associated autoantibodies may cause disease symptoms, be neutral or protect against disease. Improved understanding of these aspects of adaptive immunity in RA provides new opportunities for prevention and therapy.

Protective – or not involved at all in the disease process. The focus in this section is on immunity toward antigens that are posttranslationally modified by citrullination, but also a series of other adaptive immunities will be discussed more briefly as they may provide alternative ways of triggering or protecting against arthritis.

ANTICITRULLINE AND OTHER SPECIFIC IMMUNITIES ASSOCIATED WITH RHEUMATOID ARTHRITIS

Since the original description of antibodies to citrullinated antigens in a subgroup of RA patients [7,8], it has become clear that citrullinated epitopes of a large number of autoantigens as well as antigens derived from microorganisms can be recognized by antibodies that are reasonably specific for RA. Thus, a fast-growing literature has demonstrated antibodies in RA patients against citrullinated extracellular and/or cell surface bound ubiquitous molecules such as fibrinogen, vimentin and alpha-enolase (see review [9]), against cartilage-specific molecules such as collagen II [10], against stress proteins such as BiP [11] against enzymes such as peptidylarginine deiminases (PADs) that mediate citrullination [12] and intracellular molecules such as histones [13] and also to GPI [14]. Also reactivities against bacterial-derived molecules, such as porphyromonas gingivalis-derived enolase and PADs, have been observed [15,16]. More recently, antibodies also against other posttranslationally modified antigens, such as carbamylated (homocitrullination of lysine) proteins have been described [16\textsuperscript{*}]. Antibodies to the different citrullinated and carbamylated antigens show a certain degree of cross-reactivity, and with more extensive studies [17,18\textsuperscript{*}] a complex pattern is now emerging with some antibodies being broadly cross-reactive between different citrullinated and some carbamylated antigens, others being much more ‘private’. These observations thus add to previous knowledge of reactivities in RA against nonmodified (auto) antigens with rheumatoid factors described as early as 1939 as prime example, but also including collagen type II and other cartilage-derived molecules and GPI. In most of these cases, however, specificity for RA is less than in the case of anticitrulline immunity. Notably, additional antibody reactivities were recently described against other modifications of collagen II [19]. Taken together, these data demonstrate the tendency of the immune system to recognize posttranslationally modified self-antigens. Also, a large number of different antigens are recognized by the antibody repertoire in RA and, most likely, additional immunities will be discovered.

A major current task for research into autoimmunity and RA is subsequently to understand why and how the described autoimmunities are triggered, how they relate to the pathogenesis of different subsets of RA, and how to design strategies for the detection of additional adaptive immune reactions, which may be involved in the development or protection against RA. Below follow some comments on recent progress in these areas.

INDUCTION AND REGULATION OF RA-RELATED ADAPTIVE IMMUNITY

A key observation concerning the relationship between adaptive immunity and RA is that most of the antibody reactivities described above, including Anti-citrullinated protein antibodies (ACPAs) as well as antibodies to carbamylated antigens, rheumatoid factors and other nonmodified antigens occur before the onset of disease [20,21\textsuperscript{*}], and in most individuals the autoantibody repertoire is developed already at the onset of disease, with very few individuals becoming autoantibody positive later during the disease course [22]. Recent studies [23,24\textsuperscript{*},25\textsuperscript{*}] using different multiplex technologies have shown that these autoantibodies emerge gradually, with a clear spreading of reactivities from one initially recognized epitope toward reactivities to many different epitopes closer to disease onset. So far, no consistent pattern of timing of the appearance of antibodies has been seen, and we therefore have few clues as to any common ‘original initiating
PAD4 antibodies are produced [32] for increased citrullination at sites where the anti-PAD4, thereby creating a positive feedback system. PAD4, greatly enhance the enzymatic activity of that antibodies that cross-react with PAD3 and certain citrullinated antigens, was the recognition particularly interesting and recently described different specific RA-associated immune reactions. A need to characterize the T-cell repertoire in the initiating organs, such as the lung in RA, as well as in target organs, such as the joint, while keeping an open mind to the possibility that T-cell regulation and T-cell targets may differ from classical autoantigens. Thus, it is feasible that the regulation of immunity to posttranslationally modified self-antigens may involve T cells that are not subject to the same restrictions as T-cells reactive with nonmodified self-antigens. As illustrated by the so far described reactivities to P. gingivalis-derived molecules [15,16], we also have to consider microbes and
possibly other external agents (cf. gluten) in our search for triggers of arthritis-related immunity including anticitrulline antibodies. Furthermore, it is feasible that other ‘nonconventional’ regulatory mechanisms, such as the antibody-mediated enhancement of enzymatic activities previously mentioned [32**], may additionally contribute to the formation of pathogenic autoimmunity, and that we may consider citrulline autoimmunity as one example of an ‘immunological accident’. In such a scenario, it is no surprise that the ‘accident’ involves the generation of immunity to several different citrullinated proteins. A key issue in such a situation is to understand which of these immunities may be pathogenic, neutral or protective in relation to different disease symptoms.

**EFFECTOR AND PROTECTOR FUNCTIONS OF AUTOIMMUNITY, IN PARTICULAR ANTICITRULLINE ANTIBODIES, IN THE CONTEXT OF ARTHRITIS-RELATED SYMPTOMS**

As described above, there are now a large number of autoantibodies with different specificities described in patients with RA, and thus a number of different possibilities whereby such antibodies – as single antibodies or in combinations, may be involved in causing different symptoms associated with RA. Comments, here, will be restricted to effects of antibodies, acknowledging that the less well known T-cell reactivities may be crucial not only for help to antibody production but also in directly contributing to disease symptoms. Furthermore, we will not discuss the important potential of immunoglobulin-expressing B cells to function as antigen-presenting cells for T cells.

There is now increasing evidence that immune reactions against citrullinated and other antigens is generated locally in joints during arthritis, for example within but probably not restricted to local germinal center-like formations that are common in antibody-positive RA cases (see review [36]). Local antibody production has been demonstrated for a series of citrullinated antigens as well as for collagen II and rheumatoid factors (see reviews in [28,29]). A notable recent addition to this concept has emerged from studies on specificity and structure of monoclonal IgGs generated from single synovial fluid-derived B cells. Here, between 16 and 33% of all CD19+ IgG+ B cells from synovial fluid were shown to produce antibodies specific for different citrullinated antigens [37†]. These antibodies had different origins (different coding genes), they had undergone multiple mutations, and the B cells producing these antibodies were thus most probably driven toward differentiation by local T cells. They displayed varying fine specificities for different citrullinated autoantigens – and were totally nonreactive with the arginine variants of tested self-peptides [37†]. This study, together with much previous evidence demonstrates that immunity that begins elsewhere, for example in the lungs, may over years progress into a situation in which these same immune reactions occur also in joints, where structures that permit local and T cell-dependent immunity are established. In the case of collagen II autoimmunity, including autoimmunity to citrullinated collagen II, obvious explanations exist as the targets are relatively joint-specific. This is not the case for the immunity to the majority of citrullinated antigens in RA patients, in which we still have no obvious explanation as to why and how immunity to citrullinated, and possibly also carbamylated, antigens sometimes focuses to the joints. Obviously, however, most individuals with anticitrulline immunity never experience this transformation to a state of arthritis, and in most individuals who develop RA, it takes years with existing systemic anticitrulline immunity before the transformation takes place.

Thus, it will still be essential to investigate in detail the ways in which different RA-associated autoimmune reactions, in particular the different anticitrulline reactions, may contribute to various disease symptoms in seropositive patients, as well as to better understand the remarkable focusing of anticitrulline immunity to joints.

As emphasized above, the best-described effects are for antibodies against collagens including citrullinated collagen type II. Thus, immunization with citrullinated collagen II is more arthritogenic (in rats) than noncitrullinated collagen II [38]. Furthermore, monoclonal mouse antibodies specifically reactive with an immunodominant epitope on citrullinated collagen II cause arthritis upon infusion in mice [4]; interestingly, antibodies against this epitope are quite common in RA patients [10]. Even administration of citrullinated collagen II without adjuvant was recently described to cause arthritis [39]. It is also notable that different anticollagen II antibodies are very different concerning their disease-inducing capacity. A cocktail of several antibodies is more efficient than single antibodies, and at least antibodies against one particular epitope are protective against arthritis [40]. A few studies have indicated that also immunization with citrullinated endogenous fibrinogen [41] or bacterial-derived enolase [42] may induce arthritis; these results are of great interest and are awaiting further confirmation and extension.

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A recently described effect of ACPAs representing a potentially important mechanism in arthritis (Kleyer and Schett, pp. 80–84 of this issue) is the demonstration that antibodies against certain citrullinated antigens, including citrullinated vimentin, may cause osteoclast activation [6**]. If this direct method of activation of a single-cell type can be shown to be unique for the osteoclast, these findings have obvious implications for the understanding of an early sequence of events in seropositive RA, with the potential that initial inflammatory and osteoclast-dependent events may take place in the bone marrow and in the cartilage–bone–pannus junction. These events may hypothetically initiate a further spread of inflammation in the synovium in which other cells as well as other immune reactions may contribute to the perpetuation of inflammation.

A most obvious question is, however, whether and how the most commonly found anticitrulline antibodies in RA patients, which are directed against noncartilage self-molecules, can activate synovial inflammation in a more direct way, in addition to the effects on osteoclasts. So far, experiments aimed at directly transferring arthritis to mice with the help of anticitrulline antibodies from serum of RA patients have generally failed to directly induce arthritis (our own unpublished observations). Similarly, mouse monoclonal antibodies against citrullinated fibrinogen failed to induce arthritis upon direct transfer experiments in mice, but were able to enhance the development of arthritis when a mild synovitis had been induced with anticollagen II antibodies [43]. These observations are compatible with the fact that ACPAs may indeed exist over many years without causing disease, and that the majority of individuals with ACPAs actually do not develop arthritis. Thus, something in addition to the mere presence of these antibodies appears to be needed to eventually make these ACPAs contribute to the development of synovitis. Here, recently published studies have suggested the need for such an additional mechanism (compatible with a previously proposed ‘second signal’ [29]). Thus, ACPAs from RA patients were shown to activate the production of neutrophil extracellular traps (NETs) from neutrophils [44**]. Such NETs contain molecules needed for innate antimicrobial defense including LL37 and some of the molecules ion the NETs are indeed citrullinated [45]. In a report from Khandpur et al. [44**], these NETs were shown to have potent macrophage activating properties. It is thus feasible that the formation of NETs, possibly as a consequence of an antimicrobial defense in joints, might function as an intermediate step between ACPAs and effector functions in the joint. As neutrophil activation and thus release of NETs is a well-known feature of many joint inflammatory reactions, this may also be an additional mechanism able to focus the effector functions of anticitrulline immunity and ACPAs to joints.

Additional potential effector mechanisms along the same lines are proposed from the observation that citrullinated fibrinogen may bind to receptors, in particular toll-like receptors on fibroblasts, including synovial fibroblasts, and that antibodies to epitopes on the citrullinated fibrinogen may enhance the activation of these cells [46]. This proposed mechanism is indeed similar to the mechanism that has been proposed and widely verified in systemic lupus erythematosus (SLE), where dsDNA has been shown to bind to receptors on plasmacytoid dendritic cells and where binding of anti-dsDNA antibodies to the cell-bound dsDNA, and subsequent engagement of Fc receptors, mediate the production of alpha-interferon from these target cells for the anti-dsDNA antibodies [47].

Several additional effector mechanisms remain possible and are currently subject to active investigation in many laboratories. It is feasible that these studies will show that different antibodies may contribute to different symptoms in RA patients, and possibly explain why different patients experience different clinical problems, sometimes dominated by inflammation in joints, in other cases more associated with extra-articular manifestations (including inflammation in lungs), in other cases with very active bone destruction and in still others with pain as a major component.

**CONCLUSION**

The interesting scenario now emerging is that immune reactions, including antibodies with different fine specificities, may contribute to different RA-related symptoms, and also that the presence of antibodies with certain specificities or other unique features may in fact protect against these symptoms. If the findings summarized above are replicated, we may be able both to predict disease development and symptomatology with the help of antibody analysis, and possibly also influence disease course by the administration of antibodies with certain features. Such protective effects may depend both on specificity as suggested both from previous experiments with anticollagen antibodies [40] and from a recent study [48] on effects of monoclonal antibodies reactive with different citrullinated peptides. Protective effects may thus depend on specificity but may also depend on other features, in particular glycosylation. A particularly interesting
example of effects of different glycosylation patterns have been provided from studies, on antibodies that have been modified by enzymes from bacteria, in which these enzymes appear to have evolved during evolution to modify IgG antibodies so that they do not bind complement or Fc receptors [49]. Thus, antibodies with glycosylation modified by one of these enzymes, EndoS, are not able to exert any effector functions in arthritis, and may instead be able to protect against disease [49]. A very recent study [50**] indicates, that such deglycosylated IgG molecules may indeed inhibit immune complex dependent inflammation even when the deglycosylated antibodies are not directed to the targets of the pathogenic antibodies. But complexity does not stop here. It appears that also ACPAs other than those of the IgG class may have important effects on arthritis. Thus IgE ACPAs have been suggested to be critical for the degranulation of mast cells in joints, and therefore contribute to the development of synovitis [51]. In addition, IgA ACPAs have been described and suggested to mediate protective effects [52].

The present review summarizes a number of features that make seropositive RA a very interesting 'prototypic disease' for analysis and manipulation of antibody-dependent inflammation, and some of these features associated with potential effector functions are summarized in the list below. Tentative effector functions of ACPA are:

1. Osteoclast activation [6**]: ACPA production in response to certain citrullinated proteins, mainly vimentin may induce bone loss, providing a link between the adaptive immune system and bone.

2. Activation of NETs production from neutrophils [44**]: As neutrophil activation and thus release of NETs is a feature of many inflammatory reactions in joints, this may be an additional mechanism able to focus the effector functions of anticitrulline immunity and ACPAs to joints. It has been suggested that externalization of citrullinated epitopes and immunostimulatory molecules contributes to the NETosis, which may promote aberrant adaptive and innate immune responses in the joint, and therefore perpetuate pathogenic mechanisms in this disease.

3. Macrophages activation [46]: Antibodies against citrullinated fibrinogen may enhance the activation of macrophages by binding to their receptors, in particular toll-like receptors.

4. ACPA may have protective effects depending on the specificity and/or other features, mainly glycosylation of their Fc parts. Thus, antibodies with certain glycosylation patterns are not able to exert any effector functions in arthritis, and may instead be able to protect against disease [49].

5. Mast cells degranulation: it has been suggested by Schuerwegh et al. [51] that IgE ACPAs are critical for the degranulation of mast cells in joints and therefore contribute to the development of synovitis, and also IgA ACPAs have been described and suggested to mediate protective effects.

These features include a gradual emergence of several different disease-specific antibodies, the presence of such antibodies long before joint symptoms, production of at least some of these antibodies in initiator organs such as the lungs as well as in effector organs such as the joint and access to experimental systems where effector as well as protective effects of these antibodies can be analysed. The situation is thus very attractive for testing of interventions in the various defined phases of RA development (see [53]). It is our hope, therefore, that the rapid advancement of our understanding of adaptive and specific immunity in cause and pathogenesis will soon evolve into a new phase in which a series of interventions can be tested for their abilities to prevent or treat major subpopulations of RA.

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Conflicts of interest
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REFERENCES AND RECOMMENDED READING
Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest


This article describes how antibodies to citrullinated II antibodies of the same fine specificity as present in some RA patients can induce pain in mice.


This article describes how certain ACPAs including those with reactivity to citrullinated vimentin peptides can activate osteoclasts and promote development of bone erosion.


This article defines a new and functionally interesting cellular component as a target for ACPAs and complements reference [44].


This article describes a classical molecular mimicry situation with autofiltrullination of the citrullinating enzyme in P. gingivalis bacteria, and potentials for cross-reactivity with a human PAD counterpart.


This article describes antibodies to carbamyalted antigens, and certain features of their cross-reactivities.


This article describes how modifiable factors have a major influence on which ACPA-positive individuals with arthralgia will progress to rheumatoid arthritis.


This article provides additional evidence that different mechanisms – genetic and environmental – are involved in triggering production of ACPAs with different fine specificities.


This article demonstrates a new feed-forward mechanism for the development of ACPAs by showing that certain antibodies against the citrullinating enzymes PAD3 and PAD4 greatly enhance the citrullinating capacity of PAD4.


Ireland JM, Unanue ER. Processing of proteins in autophagy vesicles of antigen-presenting cells generates citrullinated peptides recognized by the immune system. Autophagy 2012; 8:429–430.

This article shows that autophagy is associated with citrullination of certain endogenous proteins, and provides therefore a new potential mechanism for activation of anticitrulline immunity.


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This article provides important data on the emergence of the ACPA repertoire in individuals prior to development of rheumatoid arthritis.


This article provides important data on the emergence of the ACPA repertoire before onset of RA.


This article provides additional data from a very large cohort of pre-RA patients concerning the emerging repertoire of ACPAs before the onset of disease. Increase in frequency and titers of a subset of specific ACPAs is described before disease onset.


This article describes how smoking and overweight are involved in triggering production of ACPAs with different fine specificities.


Ireland JM, Unanue ER. Processing of proteins in autophagy vesicles of antigen-presenting cells generates citrullinated peptides recognized by the immune system. Autophagy 2012; 8:429–430.

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This article provides important data on the emergence of the ACPA repertoire in individuals prior to development of rheumatoid arthritis.
This article describes how ACPAs may stimulate formation of NETs and that such NETs may activate macrophages to a more inflammatory state. The findings provide a new potential link between presence of ACPAs and activation of proinflammatory events in the joint.
This article provides evidence that EndoS-treated IgG can inhibit IgG immune complex driven joint inflammation via a new mechanisms that involves interference with immune complex formation. If confirmed and expanded to other conditions and models, these findings open new potentials for interference with immune-complex-mediated diseases.