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Review Article

The roles of anti-citrullinated protein antibodies in the immunopathogenesis of rheumatoid arthritis

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Abstract

Rheumatoid arthritis (RA) is a common systemic autoimmune disease. Its major manifestation is persistent joint inflammation, which can lead to bone destruction and severe disability. The immunopathogenesis of RA is very complex, involving both innate and adaptive immune systems. Recently, the discovery of anti-citrullinated protein antibodies (ACPAs) has revolutionized the diagnosis and our understanding of the immunopathogenesis of RA. The presence of ACPAs is also closely linked to the disease activity of RA. Therefore, it is reasonable to believe that ACPAs and protein citrullination are key issues for the development of RA. We have summarized the recent study results in this review. The first theory concerning the pathogenesis of RA proposed that ACPAs link the well-known genetic and environmental risk factors for developing RA. However, due to the close association between joint inflammation and ACPAs, a more direct role of ACPAs in the immunopathogenesis of RA is anticipated. Within the past 10 years, many studies, including some of our own, have shown that ACPAs can promote an inflammatory response through complement activation, formation of neutrophil extracellular traps, and direct binding to key players, including monocytes, osteoclasts, and osteoblasts, in the mediation of bone destruction in the joints of RA patients. We also present some new perspectives and issues that need to be further investigated.

Keywords: *Anti-citrullinated protein antibodies, Citrullination, Peptidylarginine deiminase, Rheumatoid arthritis*

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Introduction

Rheumatoid arthritis (RA) is a common systemic autoimmune disease characterized by chronic inflammation of the joints. The chronic joint inflammation can induce the formation of pannus tissue and ultimately leads to joint destruction [1]. In addition, patients with RA can develop extra-articular manifestations, such as interstitial lung diseases, vasculitis, and systemic comorbidities, including cardiovascular disease, osteoporosis, and diabetes. These conditions can lead to an increased risk of mortality in patients with RA [2]. RA affects around 1% of the population with a female-to-male ratio of approximately 2.5–1. The incidence of RA increases with age and it most commonly affects women aged 40–60 years [3].

The pathogenesis of RA is very complex, involving both innate and adaptive immunity. B-cell abnormality with the presence of autoantibodies, leading to the formation of immune complexes, aberrant T-cell responses, proinflammatory and anti-inflammatory cytokine imbalance, and aggressive tumor-like features of the rheumatoid synovium are well-known mechanisms in the pathogenesis of RA [4]. The aim of this review is to summarize recent advances in the roles of anti-citrullinated protein antibodies (ACPAs) in the immunopathogenesis of RA.

Anti-citrullinated protein antibodies

Formerly, the presence of rheumatoid factor (RF) in the patient's serum was the most important biomarker for the diagnosis of RA [5]. However, RF can also be detected in the sera from patients with other rheumatic diseases, such as primary Sjögren's syndrome (pSjS), systemic lupus erythematosus (SLE), dermatomyositis, polymyositis, and progressive systemic sclerosis [6]. Our previous study also showed a high RF positivity rate in the serum of patients with pSjS, chronic hepatitis B infection, and hepatitis C infection [7].

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In 1964, Nienhuis *et al*. reported the presence of antiperinuclear factor (APF), which is an autoantibody against human keratohyalin granules of buccal mucosa cells, in the serum of patients with RA [8]. Later, in 1979, Young *et al*. discovered an autoantibody that reacted with the keratinized tissue of the rat esophagus (AKA) in the serum of patients with RA [9]. Both APF and AKA were highly specific for the diagnosis of RA. At the end of the 20th century, two groups of scientists found that the cognate antigen for APF and AKA was the citrullinated, but not the native form, of filaggrin [10,11]. Citrullination is a posttranslational modification (PTM) of protein that is catalyzed by peptidylarginine deiminase (PADI) in the presence of a high concentration of calcium. Protein citrullination occurs widely in cell differentiation, inflammatory responses, cell apoptosis, gene regulation, and aging process. Several proteins, including vimentin, fibrin, and α-enolase, have been found to be citrullinated, and they can then be recognized by ACPAs. The citrullination of protein causes loss of basic charge(s), which can influence the protein structure and create a new epitope recognized by the immune system [12,13]. The presence of ACPAs in the serum is now the most specific biomarker for the diagnosis of RA [14]. The presence of ACPAs can predict the development of RA in patients with early, undifferentiated arthritis [15]. ACPAs are also present in RA sera several years before a definite diagnosis of RA [16,17]. In RA patients, the presence of ACPAs has been associated with active inflammation and subsequent destruction and deformity of the joints [18,19]. In 2010, 12 years after the identification of ACPAs, the American College of Rheumatology/European League Against Rheumatism revised their classification criteria to include the presence of ACPAs in the diagnosis of RA [20].

Role of anti-citrullinated protein antibodies in the pathogenesis of rheumatoid arthritis

A first theory for how ACPAs and protein citrullination could participate in the pathogenesis of RA came from observation of gene–environment interaction in RA patients. Smoking and carrying certain HLA-DR alleles (HLA-DR SE alleles) are both known risk factors for developing RA. Smoking was also found to be a risk factor for RA in RA patients with ACPAs in their serum (ACPA (+) RA) but had no effect in RA patients without ACPAs in their serum (ACPA (-) RA). HLA-DR SE alleles and smoking dramatically and synergistically increase the risk of developing RA in ACPA (+) RA patients, but not ACPA (−) RA patients [21]. Smoking can enhance PADI expression in the bronchial mucosal and alveolar compartment and facilitate the generation of citrullinated proteins [22]. HLA-DR SE alleles can bind citrulline at its Class II MHC anchor positions, and the conversion of arginine to citrulline by PADI can increase the binding affinity. Citrullination of protein can also alter the protein structure and create new epitopes. T cells recognizing these new peptides would be expected to escape from the negative selection and thus create a pool of autoreactive cells [23]. Besides the above molecular mechanism focusing on protein citrullination, the clinical association of ACPAs and the disease activity of RA mentioned previously suggest that ACPAs could play a more direct role in the immunopathogenesis of RA. The first line of evidence came from animal studies. In mice with collagen-induced arthritis, passive transfer of ACPAs aggravated joint inflammation [24,25]. Therefore, ACPAs should directly contribute to the pathogenesis of RA. The current studies of ACPAs contributing to the inflammatory response in patients with RA are summarized below.

Complement activation and rheumatoid factor

Clavel *et al*. demonstrated that immune complexes formed by citrullinated fibrinogen and ACPAs can induce macrophages to secrete tumor necrosis factor alpha (TNF-α) through binding to Fc-gamma receptor (FcγR) IIa [26]. Subsequently, ACPAs were found to activate complement via both classical and alternative, but not leptin, pathways [27]. Sokolove *et al*. further demonstrated that in addition to FcγR, immune complex-containing citrullinated fibrinogen can also stimulate macrophages to produce TNF-α via Toll-like receptor 4 [28]. RF could enhance TNF-α, interleukin (IL)-6, and IL-8 secretion by macrophages induced by ACPAs containing immune complexes through FcγR [29].

Monocytes

In 2010, our research team found that ACPAs bind to cell surface-expressed citrullinated 78 kDa glucose-regulated protein (GRP78) on U937 cells, a monocytic cell line, then activate nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), and enhance the secretion of TNF-α [30]. Later, it is found that ACPA (+) RA patients have increased phosphorylation of extracellular signal-regulated kinase (ERK) and c-Jun N-terminal kinase (JNK), but not P38, in synovial tissue compared with ACPA (−) RA patients [31]. Our *in vitro* study showed that ACPAs can selectively activate the ERK and JNK signaling pathways to enhance Akt and IκB kinase α phosphorylation, which leads to activation of NF-κB and production of TNF-α [32]. Recently, we showed that ACPAs led to decreased expression of let-7a, an microRNA, in monocytes from ACPA (+) RA patients. The decreased expression of let-7a can enhance ACPA-mediated phosphorylation of ERK and JNK and increase expression of IL-1β through increased expression of Ras proteins [33]. In addition to facilitating the inflammatory pathway of RA, decreased let-7a expression has been associated with RF positivity in ACPA (+) RA patients.

Osteoclasts

The cardinal manifestation of RA is severe joint destruction and generalized bone loss. Increased osteoclast differentiation and decreased recruitment and differentiation of osteoblast progenitors can aggravate joint damage and systemic bone loss [34]. In 2012, Harre *et al*. showed that ACPA (+) RA patients had higher bone resorption compared with ACPA (−) RA patients with the degree of bone resorption correlated with the ACPA titer. In animal models, ACPAs were found to promote differentiation of osteoclasts and induce bone loss through binding to surface-expressed mutated citrullinated vimentin [35]. Later, Krishnamurthy *et al*. demonstrated that increased PADI enzyme expression followed by enhanced protein citrullination was essential for osteoclast differentiation.

Thus, ACPAs could induce the activation of osteoclasts with IL-8. ACPA-induced systemic bone loss in mice could be blocked by an IL-8 antagonist [36].

Osteoblasts

The osteoblast is another key player in osteoimmunology which can mediate bone destruction in patients with RA. In 2016, we found that ACPAs could lead to apoptosis of SAOS-2 cells, a mature osteoblast cell line, via binding to cell surface-expressed citrullinated heat shock protein 60 (HSP60). Patients with RA had higher serum titers of antibodies against citrullinated HSP60, but not the native form of HSP60, compared with controls. In addition, the levels of antibodies against citrullinated HSP60 were positively associated with joint damage in patients with RA [37].

Synovial fibroblasts

The synovial fibroblast is a critical player in the formation of joint inflammation and bone destruction in RA. ACPAs have also been demonstrated to bind to citrullinated heterogeneous nuclear ribonucleoproteins A2/B1 on synovium fibroblasts. However, its effect on the function of synovial fibroblasts is unclear [38].

Neutrophil extracellular traps

Neutrophil extracellular traps (NETs) are fibrous networks composed of granule proteins and chromatin from neutrophils. They can bind pathogens such as bacteria [39] and can trigger inflammation and cell death [40]. Increased NET formation was observed in neutrophils from peripheral blood and synovial fluid of RA patients compared with those from healthy controls or patients with osteoarthritis. Citrullinated proteins are externalized during the formation of NETs, and ACPAs can enhance the formation of NETs [41]. Increased histone citrullination is found during NET formation. Histone citrullination promotes NET formation by enhancing chromatin decondensation and inducing the expulsion of DNA [42]. Since PADI4 is essential for NET formation, so inhibiting PADI4 could reduce NET formation [43]. The formation of NETs also generates citrullinated antigens, which could be potential targets for ACPAs, and thus connects innate and adaptive immunity in RA [44]. Furthermore, B cells isolated from synovial tissue in RA joints can generate ACPAs targeting the citrullinated protein formed during NET formation [45].

Pain

To our surprise, antibodies from patients with RA induced pain-like behavior in mice. This behavior was specifically induced by the ACPA-containing fraction of immunoglobulin G (IgG). ACPAs can accumulate in the skin, ankle joints, and bone marrow without inducing obvious joint inflammation and neuron excitation. ACPAs can bind to osteoclasts and induce the release of the nociceptive chemokine, CXCL1 (analog to human IL-8) [46]. This pathway might explain the frequently observed disconnection between tender joints and swollen joints.

Antibody glycosylation

Carbohydrate chains on the antibodies are known to attach to both the Fc and the Fab region of antibodies, which are important for immune effector functions. The Fc region of purified ACPAs from RA patients contains a significantly lower degree of galactosylation and sialylation compared with IgG antibodies [47]. Desialylated IgG-containing immune complex stimulates osteoclast differentiation through binding to Fc receptor both *in vivo* and *in vitro*. Moreover, RA patients with low levels of ACPA-IgG Fc sialylation showed lower bone volumes and trabecular numbers. *In vitro* sialylation of ACPAs could remove their ability to promote osteoclast differentiation [48]. Strikingly, the change of glycosylation in ACPAs was not limited to the Fc part, but N-linked glycans were frequently observed in variable domains of ACPAs. The N-glycosylation sites on ACPA variable domains are formed during somatic hypermutation. This finding revealed that ACPA hyperglycosylation confers a selective advantage to B cells that produce ACPAs. The importance of this unique feature of the citrulline-specific immune response in RA deserves further study [49].

Anti-carbamylated protein antibodies and protein carbamylation

Carbamylation is a nonenzymatic PTM of protein with cyanate. Low-grade carbamylation occurs under normal steady-state conditions but might be enhanced under uremia, smoking, and inflammatory conditions. For example, the action of neutrophil-releasing myeloperoxidase increases local levels of cyanate and therefore facilitates protein carbamylation. Carbamylation can modify the N-terminus of proteins on amino acids, including arginine and cysteine, but mostly in the conversion of lysine to a homocitrulline. Homocitrulline resembles citrulline but is one CH_2 group longer than citrulline [50,51].

Anti-carbamylated protein (anti-CarP) antibodies can be detected in about 16% of seronegative RA patients. Their presence is correlated with more severe joint damage [52] and represents a risk factor for developing RA in patients with inflammatory arthralgia [53]. Anti-CarP antibodies can also be detected in the sera of RA patients before clinical manifestation of RA [52]. Therefore, they can be used as a tool for the diagnosis and follow-up of RA. Although anti-CarP antibodies and ACPAs share some similarity and cross-reactivity [54], they also exhibit some differences. The presence of anti-CarP antibodies does not correlate with previously known HLA-SE alleles or protein tyrosine phosphatase, nonreceptor type 22 (PTPN22) polymorphisms, the two important genetic risk factors for ACPA (+) RA patients, or with smoking, a well-known environmental risk factor for developing RA. In contrast, the presence of anti-CarP antibodies is generally associated with the HLA-DRB1_03 haplotype [55]. In 2016, a study from our research group demonstrated that the serum titer of anti-carbamylated GRP78 antibody was significantly elevated in patients with RA. However, anti-carbamylated GRP78 antibody is also frequently detected in patients with SLE or pSjS [56]. Therefore, ACPAs are still the most specific biomarker to date, and the potential biologic functions of anti-CarP antibodies require further investigation.

We have summarized the cells and molecular and clinical mechanisms targeted by ACPAs and the respective processes related to the pathogenesis of RA in Table 1.

Table 1: Summary of direct targets of anti-citrullinated protein antibodies and their effects in the immunopathogenesis of rheumatoid arthritis

Target	Regulated process	Reference(s)
Complement system	Induce macrophages to secrete TNF-α via Fc receptor and TLR4	[26-28]
	Activate classical and alternative complement pathways	
Monocytes	Activate ERK and JNK pathways, leading to activation of NF-κB and production of TNF-α	[30,32,33]
	Decrease expression of let-7a, increase expression of Ras proteins, and increase IL-1β secretion	
Osteoclasts	Promote differentiation of osteoclasts and induce bone loss	[35,36]
	Increased PADI enzyme expression is essential for osteoclast differentiation; ACPA-induced systemic bone loss is via induction of IL-8	
Osteoblasts	Promote osteoblast apoptosis	[37]
Synovial fibroblasts	Bind to citrullinated protein on synovium fibroblasts. Functional effect is unclear	[38]
NETs	Enhance formation of NETs and generate citrullinated antigens	[41,44]
Pain	Induce pain-like behavior in mice	[46]

ACPAs: Anti-citrullinated protein antibodies, TNF-α: Tumor necrosis factor-α, TLR4: Toll-like receptor 4, ERK: Extracellular signal-regulated kinase, JNK: c-Jun N-terminal kinases, NF-κB: Nuclear factor kappa-light-chain-enhancer of activated B-cells, IL: Interleukin, PADI: Peptidylarginine deiminase, NETs: Neutrophil extracellular traps

Future prospective

RA patients have a higher mortality risk compared with healthy controls and their higher cardiovascular mortality cannot be explained by traditional cardiac risk factors. The presence of ACPAs in the sera has been associated with increased cardiovascular death in patients with RA [57]. Citrullinated proteins have been found in the myocardium of patients with RA [58] and in atherosclerotic plaques from non-RA patients [59]. The potential effect of ACPAs on endothelial function and its binding antigen should be further examined.

In addition to ACPAs, our studies found increased GRP78 citrullination in the peripheral blood mononuclear cells (PBMCs) of patients with RA [32]. Chang *et al*. showed hypercitrullinaton of histones in the PBMCs of patients with RA as well as first-degree relatives of RA patients. The protein hypercitrullination in PBMCs of these individuals was associated with increased secretion of IL-2 and T-helper (Th) 17 cytokines but decreased secretion of Th2 cytokines. This abnormality is believed to be caused by impaired transcription of PTPN22, a phosphatase that inhibits protein citrullination [60].

Both protein citrullination and carbamylation belong to PTMs of proteins, one of the hallmarks of aging and age-related diseases [61]. ACPAs and anti-CarP antibodies belong to a group of antibodies called "anti-modified protein antibodies (AMPAs)." In fact, protein oxidation, acetylation, and antibodies against oxidized and acetylated proteins are all known to present in patients with RA [62]. The specificity of some newly found AMPAs for the diagnosis of RA and their roles in the immunopathogenesis of RA remains largely unknown at present. The wide presence of AMPAs brings forth a few questions that should be addressed: Do patients with RA have excessive formation of protein PTM related to premature aging? Do these patients have some defects in clearing proteins after PTM? Do RA patients have immune dysregulation that can lead to the formation of AMPAs? By answering these questions, we may gain a better understanding of the development of RA and also advance our concepts of aging and immunotolerance.

Conclusion

ACPAs are not only an important diagnostic marker for the classification of RA but also involve directly in the immunopathogenesis of RA through the facilitation of NET formation, ligation to Fc receptors, and direct modulation of the functions of monocytes, osteoclasts, and osteoblasts. Several critical questions need to be answered is to fully elucidate the role of ACPAs in the immunopathogenesis of RA.

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Conflicts of interest

There are no conflicts of interest.

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