Tzu Chi Medical Journal 2019; 31(1): 5–10

Review Article

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

Hui-Chun Yu[a], *Ming-Chi Lu*[a,b]*

[a]Division of Allergy, Immunology and Rheumatology, Dalin Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation, Chiayi, Taiwan, [b]School of Medicine, Tzu Chi University, Hualien, Taiwan

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*

Received : 28-Jun-2018
Revised : 12-Jul-2018
Accepted : 19-Jul-2018

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].

Access this article online

Quick Response Code:

Website: www.tcmjmed.com

DOI: 10.4103/tcmj.tcmj_116_18

*Address for correspondence:
Dr. Ming-Chi Lu,
Division of Allergy, Immunology and Rheumatology,
Dalin Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation,
2, Min-Sheng Road, Dalin, Chiayi, Taiwan.
E-mail: e360187@yahoo.com.tw

How to cite this article: Yu HC, Lu MC. The roles of anti-citrullinated protein antibodies in the immunopathogenesis of rheumatoid arthritis. Tzu Chi Med J 2019;31(1):5-10.

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.

Acknowledgments

We thank Dr. Malcolm Koo for his writing assistance.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Lee DM, Weinblatt ME. Rheumatoid arthritis. Lancet 2001;358:903-11.
2. Gabriel SE, Crowson CS, Kremers HM, Doran MF, Turesson C, O'Fallon WM, et al. Survival in rheumatoid arthritis: A population-based analysis of trends over 40 years. Arthritis Rheum 2003;48:54-8.
3. Smolen JS, Aletaha D, McInnes IB. Rheumatoid arthritis. Lancet 2016;388:2023-38.
4. Firestein GS. Evolving concepts of rheumatoid arthritis. Nature 2003;423:356-61.
5. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988;31:315-24.
6. Percy JS, Russell AS. Laboratory diagnosis and monitoring of rheumatologic diseases. Can Med Assoc J 1975;112:1320-8.
7. Lu MC, Hsieh SC, Lai NS, Li KJ, Wu CH, Yu CL, et al. Comparison of anti-agalactosyl IgG antibodies, rheumatoid factors, and anti-cyclic citrullinated peptide antibodies in the differential diagnosis of rheumatoid arthritis and its mimics. Clin Exp Rheumatol 2007;25:716-21.
8. Nienhuis RL, Mandema E. A new serum factor in patients with rheumatoid arthritis; the antiperinuclear factor. Ann Rheum Dis 1964;23:302-5.
9. Young BJ, Mallya RK, Leslie RD, Clark CJ, Hamblin TJ. Anti-keratin antibodies in rheumatoid arthritis. Br Med J 1979;2:97-9.
10. Schellekens GA, de Jong BA, van den Hoogen FH, van de Putte LB, van Venrooij WJ. Citrulline is an essential constituent of antigenic determinants recognized by rheumatoid arthritis-specific autoantibodies. J Clin Invest 1998;101:273-81.
11. Girbal-Neuhauser E, Durieux JJ, Arnaud M, Dalbon P, Sebbag M, Vincent C, et al. The epitopes targeted by the rheumatoid arthritis-associated antifilaggrin autoantibodies are posttranslationally

generated on various sites of (pro) filaggrin by deimination of arginine residues. J Immunol 1999;162:585-94.
12. Yamada R. Peptidylarginine deiminase type 4, anticitrullinated peptide antibodies, and rheumatoid arthritis. Autoimmun Rev 2005;4:201-6.
13. György B, Tóth E, Tarcsa E, Falus A, Buzás EI. Citrullination: A posttranslational modification in health and disease. Int J Biochem Cell Biol 2006;38:1662-77.
14. Schellekens GA, Visser H, de Jong BA, van den Hoogen FH, Hazes JM, Breedveld FC, et al. The diagnostic properties of rheumatoid arthritis antibodies recognizing a cyclic citrullinated peptide. Arthritis Rheum 2000;43:155-63.
15. van Gaalen FA, Linn-Rasker SP, van Venrooij WJ, de Jong BA, Breedveld FC, Verweij CL, et al. Autoantibodies to cyclic citrullinated peptides predict progression to rheumatoid arthritis in patients with undifferentiated arthritis: A prospective cohort study. Arthritis Rheum 2004;50:709-15.
16. Nielen MM, van Schaardenburg D, Reesink HW, van de Stadt RJ, van der Horst-Bruinsma IE, de Koning MH, et al. Specific autoantibodies precede the symptoms of rheumatoid arthritis: A study of serial measurements in blood donors. Arthritis Rheum 2004;50:380-6.
17. Edwards CJ, Cooper C. Early environmental factors and rheumatoid arthritis. Clin Exp Immunol 2006;143:1-5.
18. Kastbom A, Strandberg G, Lindroos A, Skogh T. Anti-CCP antibody test predicts the disease course during 3 years in early rheumatoid arthritis (the swedish TIRA project). Ann Rheum Dis 2004;63:1085-9.
19. Syversen SW, Gaarder PI, Goll GL, Ødegård S, Haavardsholm EA, Mowinckel P, et al. High anti-cyclic citrullinated peptide levels and an algorithm of four variables predict radiographic progression in patients with rheumatoid arthritis: Results from a 10-year longitudinal study. Ann Rheum Dis 2008;67:212-7.
20. Aletaha D, Neogi T, Silman AJ, Funovits J, Felson DT, Bingham CO 3rd, et al. 2010 rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. Ann Rheum Dis 2010;69:1580-8.
21. Klareskog L, Rönnelid J, Lundberg K, Padyukov L, Alfredsson L. Immunity to citrullinated proteins in rheumatoid arthritis. Annu Rev Immunol 2008;26:651-75.
22. Makrygiannakis D, Hermansson M, Ulfgren AK, Nicholas AP, Zendman AJ, Eklund A, et al. Smoking increases peptidylarginine deiminase 2 enzyme expression in human lungs and increases citrullination in BAL cells. Ann Rheum Dis 2008;67:1488-92.
23. James EA, Moustakas AK, Bui J, Papadopoulos GK, Bondinas G, Buckner JH, et al. HLA-DR1001 presents "altered-self" peptides derived from joint-associated proteins by accepting citrulline in three of its binding pockets. Arthritis Rheum 2010;62:2909-18.
24. Kuhn KA, Kulik L, Tomooka B, Braschler KJ, Arend WP, Robinson WH, et al. Antibodies against citrullinated proteins enhance tissue injury in experimental autoimmune arthritis. J Clin Invest 2006;116:961-73.
25. Ge C, Tong D, Liang B, Lönnblom E, Schneider N, Hagert C, et al. Anti-citrullinated protein antibodies cause arthritis by cross-reactivity to joint cartilage. JCI Insight 2017;2:e93688.
26. Clavel C, Nogueira L, Laurent L, Iobagiu C, Vincent C, Sebbag M, et al. Induction of macrophage secretion of tumor necrosis factor alpha through fcgamma receptor IIa engagement by rheumatoid arthritis-specific autoantibodies to citrullinated proteins complexed with fibrinogen. Arthritis Rheum 2008;58:678-88.
27. Trouw LA, Haisma EM, Levarht EW, van der Woude D, Ioan-Facsinay A, Daha MR, et al. Anti-cyclic citrullinated peptide antibodies from rheumatoid arthritis patients activate complement via both the classical and alternative pathways. Arthritis Rheum 2009;60:1923-31.
28. Sokolove J, Zhao X, Chandra PE, Robinson WH. Immune complexes containing citrullinated fibrinogen costimulate macrophages via toll-like receptor 4 and fcγ receptor. Arthritis Rheum 2011;63:53-62.
29. Laurent L, Anquetil F, Clavel C, Ndongo-Thiam N, Offer G, Miossec P, et al. IgM rheumatoid factor amplifies the inflammatory response of macrophages induced by the rheumatoid arthritis-specific immune complexes containing anticitrullinated protein antibodies. Ann Rheum Dis 2015;74:1425-31.
30. Lu MC, Lai NS, Yu HC, Huang HB, Hsieh SC, Yu CL, et al. Anti-citrullinated protein antibodies bind surface-expressed citrullinated grp78 on monocyte/macrophages and stimulate tumor necrosis factor alpha production. Arthritis Rheum 2010;62:1213-23.
31. de Launay D, van de Sande MG, de Hair MJ, Grabiec AM, van de Sande GP, Lehmann KA, et al. Selective involvement of ERK and JNK mitogen-activated protein kinases in early rheumatoid arthritis (1987 ACR criteria compared to 2010 ACR/EULAR criteria): A prospective study aimed at identification of diagnostic and prognostic biomarkers as well as therapeutic targets. Ann Rheum Dis 2012;71:415-23.
32. Lu MC, Lai NS, Yin WY, Yu HC, Huang HB, Tung CH, et al. Anti-citrullinated protein antibodies activated ERK1/2 and JNK mitogen-activated protein kinases via binding to surface-expressed citrullinated GRP78 on mononuclear cells. J Clin Immunol 2013;33:558-66.
33. Lai NS, Yu HC, Yu CL, Koo M, Huang HB, Lu MC, et al. Anti-citrullinated protein antibodies suppress let-7a expression in monocytes from patients with rheumatoid arthritis and facilitate the inflammatory responses in rheumatoid arthritis. Immunobiology 2015;220:1351-8.
34. Schett G, David JP. The multiple faces of autoimmune-mediated bone loss. Nat Rev Endocrinol 2010;6:698-706.
35. Harre U, Georgess D, Bang H, Bozec A, Axmann R, Ossipova E, et al. Induction of osteoclastogenesis and bone loss by human autoantibodies against citrullinated vimentin. J Clin Invest 2012;122:1791-802.
36. Krishnamurthy A, Joshua V, Haj Hensvold A, Jin T, Sun M, Vivar N, et al. Identification of a novel chemokine-dependent molecular mechanism underlying rheumatoid arthritis-associated autoantibody-mediated bone loss. Ann Rheum Dis 2016;75:721-9.
37. Lu MC, Yu CL, Yu HC, Huang HB, Koo M, Lai NS, et al. Anti-citrullinated protein antibodies promote apoptosis of mature human saos-2 osteoblasts via cell-surface binding to citrullinated heat shock protein 60. Immunobiology 2016;221:76-83.
38. Konig MF, Giles JT, Nigrovic PA, Andrade F. Antibodies to native and citrullinated RA33 (hnRNP A2/B1) challenge citrullination as the inciting principle underlying loss of tolerance in rheumatoid arthritis. Ann Rheum Dis 2016;75:2022-8.
39. Brinkmann V, Reichard U, Goosmann C, Fauler B, Uhlemann Y, Weiss DS, et al. Neutrophil extracellular traps kill bacteria. Science 2004;303:1532-5.
40. Delgado-Rizo V, Martínez-Guzmán MA, Iñiguez-Gutierrez L, García-Orozco A, Alvarado-Navarro A, Fafutis-Morris M, et al. Neutrophil extracellular traps and its implications in inflammation: An overview. Front Immunol 2017;8:81.
41. Khandpur R, Carmona-Rivera C, Vivekanandan-Giri A, Gizinski A, Yalavarthi S, Knight JS, et al. NETs are a source of citrullinated autoantigens and stimulate inflammatory responses in rheumatoid arthritis. Sci Transl Med 2013;5:178ra40.
42. Wang Y, Li M, Stadler S, Correll S, Li P, Wang D, et al. Histone hypercitrullination mediates chromatin decondensation and neutrophil extracellular trap formation. J Cell Biol 2009;184:205-13.
43. Li P, Li M, Lindberg MR, Kennett MJ, Xiong N, Wang Y, et al. PAD4 is essential for antibacterial innate immunity mediated by neutrophil extracellular traps. J Exp Med 2010;207:1853-62.
44. Pratesi F, Dioni I, Tommasi C, Alcaro MC, Paolini I, Barbetti F, et al. Antibodies from patients with rheumatoid arthritis target citrullinated histone 4 contained in neutrophils extracellular traps. Ann Rheum Dis 2014;73:1414-22.
45. Corsiero E, Bombardieri M, Carlotti E, Pratesi F, Robinson W,

Migliorini P, et al. Single cell cloning and recombinant monoclonal antibodies generation from RA synovial B cells reveal frequent targeting of citrullinated histones of NETs. Ann Rheum Dis 2016;75:1866-75.

46. Wigerblad G, Bas DB, Fernades-Cerqueira C, Krishnamurthy A, Nandakumar KS, Rogoz K, et al. Autoantibodies to citrullinated proteins induce joint pain independent of inflammation via a chemokine-dependent mechanism. Ann Rheum Dis 2016;75:730-8.
47. Scherer HU, van der Woude D, Ioan-Facsinay A, el Bannoudi H, Trouw LA, Wang J, et al. Glycan profiling of anti-citrullinated protein antibodies isolated from human serum and synovial fluid. Arthritis Rheum 2010;62:1620-9.
48. Harre U, Lang SC, Pfeifle R, Rombouts Y, Frühbeißer S, Amara K, et al. Glycosylation of immunoglobulin G determines osteoclast differentiation and bone loss. Nat Commun 2015;6:6651.
49. Rombouts Y, Willemze A, van Beers JJ, Shi J, Kerkman PF, van Toorn L, et al. Extensive glycosylation of ACPA-IgG variable domains modulates binding to citrullinated antigens in rheumatoid arthritis. Ann Rheum Dis 2016;75:578-85.
50. Toes RE, Huizinga TJ. Update on autoantibodies to modified proteins. Curr Opin Rheumatol 2015;27:262-7.
51. Mastrangelo A, Colasanti T, Barbati C, Pecani A, Sabatinelli D, Pendolino M, et al. The role of posttranslational protein modifications in rheumatological diseases: Focus on rheumatoid arthritis. J Immunol Res 2015;2015:712490.
52. Shi J, Knevel R, Suwannalai P, van der Linden MP, Janssen GM, van Veelen PA, et al. Autoantibodies recognizing carbamylated proteins are present in sera of patients with rheumatoid arthritis and predict joint damage. Proc Natl Acad Sci U S A 2011;108:17372-7.
53. Shi J, van de Stadt LA, Levarht EW, Huizinga TW, Toes RE, Trouw LA, et al. Anti-carbamylated protein antibodies are present in arthralgia patients and predict the development of rheumatoid arthritis. Arthritis Rheum 2013;65:911-5.
54. Scinocca M, Bell DA, Racapé M, Joseph R, Shaw G, McCormick JK, et al. Antihomocitrullinated fibrinogen antibodies are specific to rheumatoid arthritis and frequently bind citrullinated proteins/peptides. J Rheumatol 2014;41:270-9.
55. Jiang X, Trouw LA, van Wesemael TJ, Shi J, Bengtsson C, Källberg H, et al. Anti-CarP antibodies in two large cohorts of patients with rheumatoid arthritis and their relationship to genetic risk factors, cigarette smoking and other autoantibodies. Ann Rheum Dis 2014;73:1761-8.
56. Yu HC, Lai PH, Lai NS, Huang HB, Koo M, Lu MC, et al. Increased serum levels of anti-carbamylated 78-kDa glucose-regulated protein antibody in patients with rheumatoid arthritis. Int J Mol Sci 2016;17. pii: E1510.
57. Ajeganova S, Humphreys JH, Verheul MK, van Steenbergen HW, van Nies JA, Hafström I, et al. Anticitrullinated protein antibodies and rheumatoid factor are associated with increased mortality but with different causes of death in patients with rheumatoid arthritis: A longitudinal study in three European cohorts. Ann Rheum Dis 2016;75:1924-32.
58. Giles JT, Fert-Bober J, Park JK, Bingham CO 3rd, Andrade F, Fox-Talbot K, et al. Myocardial citrullination in rheumatoid arthritis: A correlative histopathologic study. Arthritis Res Ther 2012;14:R39.
59. Sokolove J, Brennan MJ, Sharpe O, Lahey LJ, Kao AH, Krishnan E, et al. Brief report: Citrullination within the atherosclerotic plaque: A potential target for the anti-citrullinated protein antibody response in rheumatoid arthritis. Arthritis Rheum 2013;65:1719-24.
60. Chang HH, Liu GY, Dwivedi N, Sun B, Okamoto Y, Kinslow JD, et al. A molecular signature of preclinical rheumatoid arthritis triggered by dysregulated PTPN22. JCI Insight 2016;1:e90045.
61. Santos AL, Lindner AB. Protein posttranslational modifications: Roles in aging and age-related disease. Oxid Med Cell Longev 2017;2017:5716409.
62. Trouw LA, Rispens T, Toes RE. Beyond citrullination: Other post-translational protein modifications in rheumatoid arthritis. Nat Rev Rheumatol 2017;13:331-9.