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Citrullinated Proteins are Arthritogenic Autoantigens in Rheumatoid Arthritis

Lazaros I Sakkas[1], George Vaiopoulos[2] and Dimitrios P Bogdanos[1]*

[1]School of Health Sciences, University of Thessaly, Larissa, Greece

[2]Medical School, National and Kapodistrian University of Athens, Greece

Abstract

Both genetic and environmental factors are implicated for the development of Rheumatoid Arthritis (RA). The association of RA with HLA-DRB1 shared epitope (SE) suggests that T cells recognize an antigen presented on HLA-DRB1SE allele. The discovery of Anti Citrullinated Peptide Antibodies (ACPAs) greatly advanced our understanding of RA pathogenesis. ACPAs, including ACPAs against peptides derived from Epstein-Barr Virus (EBV), appear many years before the onset of clinical RA and are associated with severe disease. Citrullination is the conversion of arginine residues to citrulline in proteins, mediated by Peptidyl Arginine Deiminases (PADs). Citrullination increases the peptide binding affinity to HLA-DRB1SE that can activate T cells and provide help to B cells for ACPA production and there is increased frequency of T cells recognizing citrullinated (cit) peptides in ACPA + RA. Periodontal Disease (PD), a risk factor for RA, can provide cit neoantigens. *Porphyromonas gingivalis*, a causative agent for PD, expresses PAD (PPAD), and arginine ginpains (Rgps) which preferentially cleave proteins at terminal arginine residues. Cross-reactivity was detected between ACPAs against *P. gingivalis* or EBV peptides and human peptides which can re-direct immune response to self-antigens. There is some evidence suggesting that ACPAs are arthritogenic. In animal models, cit peptides can cause or exacerbate arthritis in a HLA-DRB1SE-restricted manner. ACPAs can also activate macrophages and cause bone resorption.

Keywords: Anti-cyclic citrullinated antibodies; Autoantigen; Autoantibody; Rheumatoid arthritis

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Correspondence:
Lazaros I Sakkas, Department of Rheumatology and Clinical Immunology, Faculty of Medicine, School of Health Sciences, University of Thessaly, Larissa 41 110, Greece, Tel:+30-2413502813; Fax: +30-2413501016; E-mail: lsakkas@med.uth.gr

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Introduction

The etiology of Rheumatoid Arthritis (RA) is incompletely understood. Twin studies have shown that environmental factors rather than genetic factors appear to play a major role in the development of the disease [1]. Periodontal Disease (PD) and cigarette smoking are two environmental risk factors for RA. In the last ten or so years progress has been made that advanced our understanding on RA induction and this will be reviewed in this article.

Rheumatoid Arthritis is an Antigen-Driven Immune Disease

In the 1970s RA has been found to be associated with HLA-DR4, and this association has been expanded later to other HLA-DR alleles. All HLA-DR alleles associated with RA bear a common DR β chain sequence at positions 70-74, known as HLA-DRB1 shared epitope (HLA-DRB1*SE) [2,3]. The function of HLA-DRB1 molecules is to present antigenic peptides to T cells via the T cell antigen receptor (TCR). Therefore, this HLA-DR association plus the heavy infiltration of arthritic synovial membrane with macrophages and T cells and the increased levels of inflammatory T cell-cytokines in arthritic joints suggested that RA is a T cell-mediated disease. However, the inciting antigen was not known. The involvement of B cells attracted much less attention. Yet, patients with RA have Rheumatoid Factor (RF), an autoantibody against the Fc region of IgG, but the pathogenetic role of RF remained elusive. In RA synovial membrane there are ectopic lymphoid structures with features of germinal center and ability to present antigen and differentiate B cells into antibody-producing plasma cells. In early 2000s RA patients were found to have anti-citrullinated peptide antibodies ACPAs) and this gave a strong push to efforts for elucidating the etiopathogenesis of RA.

B-Cells Recognize Citrullinated Autoantigens: The Anti-Citrullinated Peptide Antibodies

ACPAs recognize citrullinated epitopes of various proteins. Citrullination is a post-translational

conversion of arginine to citrulline mediated by the enzymes arginine deiminases (PADs). ACPAs appear up to 12 years before clinical manifestations of RA and their frequency increases with approaching the onset of clinical RA [4,5]. In fact, these ACPAs, pre-dated the onset of RA are directed against endogenous citrullinated antigens and viral citrullinated antigens derived from Epstein-Barr virus nuclear antigen (EBNA1 and EBNA2) [6]. ACPAs appear in low levels and against one or few autoantigens at first, and their levels and specificities increase at the preclinical inflammatory phase of RA [7,8].

Cigarette smoking is a risk factor for ACPA+RA [9-11] and for PD [12]. In patients with RA, ACPAs are associated with HLA-DRB1*SE and smoking [13,14]. More interestingly, in undifferentiated arthritis, HLA-DRB1SE is a risk factor for ACPA and not an independent risk factor for the development of RA [15]. There appear to be a dosage effect among HLA-DRB1SE alleles for ACPA(+) RA. Patients carrying both HLA-DRB1*SE alleles had higher Odds Ratio for ACPA(+) RA than patients carrying just one HLA-DRB1*SE allele [16]. There is also a significant interaction between cigarette smoking and HLA-DRB1*SE for ACPA(+) RA [17-20]. Cigarette smoking is also a risk factor for RA development in pre-RA individuals [21].

ACPAs are produced in RA arthritis joints. A high percentage (~25%) of IgG-expressing B cells from ACPA(+) RA synovial fluid recognize citrullinated autoantigens [22]. Furthermore, ACPA production may be triggered by Ebstein-Barr Virus (EBV) infection. For instance, ectopic lymphoid structures from ACPA(+) RA synovial membrane transplanted into severe combined immunodeficiency (SCID) mice are able to sustain ACPA production plus anti-EBV antibodies [23].

Periodontal disease (PD) is associated with RA and this is attributed to *Porphyromonas gingivalis*, a causative agent for PD. *P. gingivalis* expresses two unique enzymes, peptidylarginine deiminase (PPAD) that citrullinates bacterial and human proteins, and arginine gingipains which are extracellular proteases cleaving proteins at arginine residues [24,25]. PPAD has specificity for C-terminal arginine and along with arginine gingipains can citrullinate many peptides from key RA autoantigens [26]. PAD and PPAD activities in periodontium are elevated in RA and non-RA patients with PD and this site may be the initial locus of ACPA production [27]. *Aggregatibacter actinomycetemcomitans*, a causative agent for PD, can cause citrullination of proteins via neutrophil membranes pore-forming leukotoxin-A, and there is evidence of *A. actinomycetemcomitans* infection in RA patients [28].

T -Cells Recognize Citrullinated Proteins

The production of ACPAs most likely require T cell help, since ACPAs undergo isotype switching (which requires T cell help), and are associated with HLA-DRB1*SE. Indeed, it has been shown that T cells recognize citrullinated peptides presented by HLA-DRB1*SE. The conversion of arginine to citrulline at a peptide side chain that interacts with the P4 pocket (aa 71 of DRβ chain) of HLA-DRB1*SE significantly increases HLA affinity and leads to activation of CD4+ T cells in DR4-IE transgenic mice [29]. Citrulline, not arginine, is accommodated in the electropositive P4 pocket of the HLA-DRB1*04:01/04 [30]. Using HLA-II tetramers, Scally et al. [30] found citrullinated vimentin- and citrullinated aggrecan-specific CD4+ T cells in peripheral blood of HLA-DRB1*04:01(+) RA patients. Naturally processed peptides from citrullinated vimentin were also recognized by T cells in ACPA+, HLA-DRB1*04:01(+) RA patients [31]. In mice, autophagy is key event involved in the generation of citrullinated peptides by antigen presenting cells to be presented to T cells [32].

Infectious Agents may Trigger ACPA Production

Bacterial infection by *P. gingivalis*, may initiate the breaking of tolerance and autoimmunity in RA. ACPAs against the immunodominant α-enolase peptide 1 (CEP1) cross-reacted with recombinant *P. gingivalis* enolase [33]. Furthermore, 20% of plasmablasts-derived antibodies from ACPA(+) RA patients were ACPAs and 63% of these ACPAs cross-reacted with outer membrane antigens and/or citrullinated enolase from *P. gingivalis* [34]. We already mentioned that EBV may trigger ACPA production in RA synovial membrane.

Citrullinated Autoantigens are Pathogenic

ACPAs are associated with extra-articular features of RA and disease severity and predict radiographic joint erosions [35-38]. Extracellular PAD levels in synovial fluid also correlate with disease activity in RA patients [39].

ACPAs promote pro-inflammatory immune responses. Immune complexes of ACPA with fibrinogen induce macrophage TNFα production [40-42] via FcγR and TLR4 [40]. ACPAs also activate complement. In fact, low avidity ACPAs are more potent activators of complement and are associated with higher rate of joint destruction [43]. RF potentiates the pro-inflammatory effects of ACPAs on complement. IgM and IgA RF amplify complement-dependent pro-inflammatory functions of ACPAs [44-46] and this has clinical ramifications. RF and ACPAs have additive effect on erosions, number and size, but RF augments erosion burden only in ACPA(+) RA patients [47]. In addition, ACPAs can promote pro-inflammatory responses through alteration of microRNAs (miRNAs). For instance, ACPAs reduced expression of let-7a miRNA in monocytic cell line and monocytes from ACPA(+) RA patients [48]. It should be noted that let-7a transfection of monocytic cell line decreases IL-1β expression [48].

Citrullinated peptides are arthritogenic in the context of HLA-DRB1SE background. In HLA-DRB1*04:01 patients with RA a large proportion of peripheral blood T cells recognizing citrullinated peptides exhibit Th1 memory phenotype producing the pro-inflammatory IFNγ [49]. Citrullinated fibrinogen induced arthritis in DR4-IE transgenic mice but not in wild-type C57BL/6 mice [50]. Furthermore, unmodified fibrinogen could not induce arthritis in DR4-IE transgenic mice [50]. However, recombinant human α-enolase and *P. gingivalis* enolase either citrullinated or uncitrullinated induced arthritis in DR4-IE transgenic mice [51]. *P. gingivalis* exacerbated murine collagen-induced arthritis that was dependent on *P. gingivalis* PPAD [52]. On the other hand, a PAD inhibitor reduced severity of collagen-induced arthritis in mice [53]. ACPAs against fibrinogen also enhanced arthritis induced by monoclonal antibodies against collage type II [54].

ACPAs can also affect bone loss. For instance, human osteoclasts express PAD which induces vimentin citrullination during osteoclastogenesis. Furthermore, ACPAs directed against mutated citrullinated vimentin causes osteoclastogenesis and bone resorption both *in vitro* and *in vivo* [55]. Osteoclastogensis and bone loss by

ACPAs are mediated through PAD enzymes and IL-8 *in vitro* and *in vivo* [56].

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