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Review

Anti-citrullinated peptides as autoantigens in rheumatoid arthritis—relevance to treatment

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ABSTRACT

Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by the presence of rheumatoid factor (RF) and anti-citrullinated protein/peptide autoantibodies (ACPAs). Citrulline derives from arginine by peptidyl arginine deiminases, and ACPAs are directed against different citrullinated antigens, including fibrinogen, fibronectin, α-enolase, collagen type II, histones. ACPAs are present in two thirds of RA patients have higher specificity than RF for RA, and are associated with joint radiographic damage and extra-articular manifestations and they are detected years before the onset clinical arthritis. Recent studies suggest that citrullinated antigens are most likely arthritogenic autoantigens in RA. ACPA production is associated with the HLA-DRB1 shared epitope (HLA-DRB1 SE) and accounts for the well-known RA-HLA-DRB1 SE association, as T cells recognize citrullinated peptides. Smoking and periodontitis, known environmental risk factors for RA promote protein citrullination and ACPA production. Cirullinated proteins are capable of inducing arthritis in transgenic mice carrying HLA-DRB1 SE genes, and ACPAs induce macrophage TNF-α production, osteoclastogenesis and complement activation. They also induce the formation of neutrophil extracellular traps (NETs). NETs, increased in RA, are a source of citrullinated autoantigens in RA and induce fibroblast interleukin-8 production. This knowledge is likely to have therapeutic implications, as there is a need of matching therapy with patient profile. Abatacept, a T cell activation modulator, is the best therapy for ACPA(+) RA patients, although clinical data are sparse at present. Rituximab, a monoclonal antibody that depletes B cells, is also the best therapy for ACPA(+) RA patients, and clinical data support this view.

Contents

1. Introduction

The etiology of rheumatoid arthritis (RA) is not known. The concordance rate (up to 15%) of RA in monozygotic twins is low but higher than that in dizygotic twins (up to 5%) which suggests that in addition to genetic factors environmental factors also play an important role for the development of the disease [1,2]. The pathogenesis of RA is incompletely understood, but T cells have long been considered as

Abbreviations: ACPA, anti-citrullinated protein/peptide autoantibodies; APC, antigen presenting cells; CCP, cyclic citrullinated peptide; HLA-DRB1 SE, HLA-DRB1 shared epitope; IFN, interferon; IL, interleukin; NET, neutrophil extracellular trap; PAD, peptidyl arginine deiminase; RA, rheumatoid arthritis; RF, rheumatoid factor; TCR, T cell receptors; TLR, Toll-like receptor; TNF, tumor necrosis factor; Treg, T-regulatory.

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main players for the development of the disease [3,4]. A T cell-centric concept of RA pathogenesis is in line with the heavy infiltration of RA synovial membrane with CD4+ T cells, the association of RA with HLA-DRB1 alleles, and the monoclonal/oligoclonal expansion of T cells from RA joints [5–9]. Because of the very large size of the T cell repertoire (2.5×10^7–1×10^8 different T cell receptors [TCRs]) [10], these clonal expansions cannot be explained by any other mechanism except that of involving proliferation and division of T cell in response to an antigenic epitope that they recognize [11].

2. HLA-DRB1 shared epitope and rheumatoid arthritis

In the late 1980s, RA was found to be associated with HLA-DRB1 alleles carrying a common amino acid sequence at positions 70–74 of the β chain, which has been known as HLA-DRB1 shared epitope (HLA-DRB1 SE) [12,13]. HLA-DRB1 SE alleles and in particular amino acids in position 70, as well as amino acids in positions 67, 71, and 74, form the important peptide binding pocket #4 in HLA-DRB1, which binds negatively-charged peptide side chains [14,15]. The HLA-DRB1 SE at positions 70–74, and in particular the Arg-Ala-Ala at position 72–74 is associated with high risk of developing RA. This risk is modulated by amino acids at positions 70–71; Gln or Arg at position 70 and Lys at position 71 confer the highest risk for developing RA [16–19]. Various researchers have tried to decipher the mechanism underlying the HLA-DRB1 SE association of RA. The shared epitope shares aminoacid homology with Epstein–Barr virus and could be a target of cross-reactive immune response by molecular mimicry. The HLA-DRB1*0401 QKRAA amino acid motif (shared epitope) is also present in the EBV glycoprotein gp110, and healthy controls with prior EBV infection have T cell recognizing the QKRAA motif [20,21]. Alternatively, HLA-DRB1 SE alleles may predispose to arthritis by shaping T cell repertoire in the thymus and activating autoreactive or deleting regulatory T cells (Tregs). In support of that transgenic mice carrying the RA-resistant HLA-DRB1*0402 had higher numbers of Tregs than mice carrying the RA-susceptible HLA-DRB1*0401 [22]. Given the well-known function of HLA-DR molecules, the RA association with HLA-DRB1 SE suggests that antigen presenting cells (APCs) carrying HLA-DRB1 SE alleles, present an arthritogenic peptide to T cells to initiate an immune response that results in a cytokine cascade with interferon(IFN)-γ, interleukin(IL)-17, tumor necrosis factor (TNF)-α, and IL-6 [3,4] (Fig. 1). What was still missing in this scenario was the identification of the arthritogenic peptide(s).

3. Citrullinated proteins as targets of B cells in rheumatoid arthritis

In the last 15 years, there has been considerable progress on the pathogenesis of RA with the discovery of anti-citrullinated protein antibodies (ACPAs). Citrulline derives from arginine by post-translational modification by peptidyl arginine deiminases (PADs). There are various isoforms of PADs, with PAD2 and PAD4 types being expressed in the RA synovium macrophages, and leukocytes, respectively [23,24]. There are few commercial tests for the detection of ACPA, including anti-cyclic citrullinated peptide (CCP) assays, and anti-mutated citrullinated vimentin test. The first generation of CCP1 assay used a cyclic derivative of a citrullinated filaggrin peptide. A second generation CCP2 and a third generation CCP3 assays contain multiple citrullinated epitopes in an attempt to increase sensitivity. It appears that CCP3 assays may have a higher sensitivity than CCP2 assays in early RA [25–29]. ACPA reactivity may be detected in non-RA sera, particularly in patients with autoimmune hepatitis and pulmonary tuberculosis, but often is due to the presence of antibodies that react with the uncitrullinated peptide target. Therefore, it is advisable that a positive CCP2 test in non-RA patients be followed by an uncitrullinated control antigen test [30,31].

ACPA are present in nearly two thirds of RA patients and are more specific than the rheumatoid factor for RA [32–34]. In an early meta-analysis, the pooled sensitivity and specificity of anti-CCP antibodies were 67% and 95%, respectively, the positive likelihood ratio was 12.46 and the negative likelihood ratio was 0.36 [35]. The respective figures for IgM rheumatoid factor were 69%, 85%, 4.86, and 0.38 [35]. ACPAs are also associated with the severity of the disease [34,36,37]. In fact, ACPAs are the strongest independent predictor of joint radiographic progression [38] and are associated with RA-interstitial lung disease [39]. ACPAs are more specific in diagnosing RA than rheumatoid factor (RF) [35] and are the best predictor of radiographic progression in early RA [33,40]. To this end, ACPAs are now included in the new classification criteria for RA [41,42] . Furthermore, ACPAs appearing up to 10 years before the onset of clinical RA [43,44], confer strong susceptibility to RA and predict the progression to RA in patients with undifferentiated arthritis [44–47]. ACPAs are directed against different citrullinated proteins, such as vimentin, histone, a-enolase, fibrinogen, fibronectin, filaggrin, collagen type II. ACPAs also recognize viral citrullinated peptides, such as citrullinated peptides derived from Epstein–Barr virus nuclear proteins EBNA1 and EBNA2 [25,48,49]. In fact, the accumulation of multiple ACPA specificities is correlated with preclinical inflammation (elevation of TNF-α, IL-6, and IFN-γ) preceding clinical arthritis [50]. Also, in the first degree relatives of RA patients without RA, the presence of >9 ACPA specificities was associated with increased risk of having >1 tender joint [51].

3.1. Citrullinated peptides as autoantigens in rheumatoid arthritis

The production of ACPAs is associated with HLA-DRB1 SE [25,44–46,49,52]. HLA-DRB1 SE alleles explain 18% of genetic variability of ACPA(+) RA but only 2.4% of ACPA(−) RA. Furthermore, HLA-DRB1 SE alleles influence the levels of ACPAs as RA patients carrying two HLA-DRB1 SE alleles have higher levels of anti-CCP antibodies than those carrying one HLA-DRB1 SE allele. In fact, the HLA-DRB1 SE is a risk factor for ACPA production and not an independent risk factor for the development of RA [49,53,54]. Cigarette smoking, which is a susceptibility and severity factor for RA [55–57], is also a strong inducer of protein citrullination. Tobacco exposure of transgenic mice carrying RA-susceptible HLA-DR alleles induces PAD [58] and is a risk factor for ACPA in RA patients carrying the HLA-DRB1 SE [59]. Periodontitis, an oral bacterial infection, is also associated with RA [60] as patients with periodontitis appear to have increased risk for RA [61,62] and inversely patients with RA have increased risk for periodontitis [63]. *Porphyromonas gingivalis*, a microbe that is the major causative agent for periodontitis, the only prokaryotic organism expressing PAD, can cause microbial and host protein citrullination [64].

ACPAs are of IgG or IgA isotype and, therefore, are most likely to require T cell help for their production. The association of ACPAs with the HLA-DRB1 SE re-enforces this concept. Recent studies have confirmed that ACPA production is T-cell-dependent. The conversion of arginine to citrulline in vimentin peptides dramatically increases the affinity of vimentin peptides for HLA-DRB1*0401 and is necessary for T cell activation [65]. Citrullinated vimentin peptides recognized by T cells from HLA-DRB1*0401 transgenic mice that were immunized with citrullinated vimentin peptides, are also recognized by T cells from ACPA(+), HLA-DRB1*04+ RA patients [66]. Similarly, the conversion of arginine to citrulline in fibrinogen peptides generated peptides that bound to pockets 4(P4), P7 and P9 of HLA-DRB1*1001, a SE allele [67]. Recently, a study by Scally et al. showed that the HLA-DRB1 SE alleles recognize citrullinated peptides in RA [68]. Citrulline was found within the electropositive P4 pocket of HLA-DRB1*04:01/04 (SE) alleles, whereas arginine interacted with electronegative P4 pocket of RA-resistant HLA-DRB1*04:02 allele. In addition, by the use of HLA-II tetramers, CD4(+) T cells recognizing citrullinated vimentin and citrullinated aggrecan were found in the peripheral blood of HLA-DRB1*04:01 RA patients and their number correlated with disease activity [68]. This piece of evidence is in line with another work showing that RA patients with ACPA exhibited very restricted TCR CDR3 length distribution in synovial biopsies, which reflected monoclonal/