Editorial

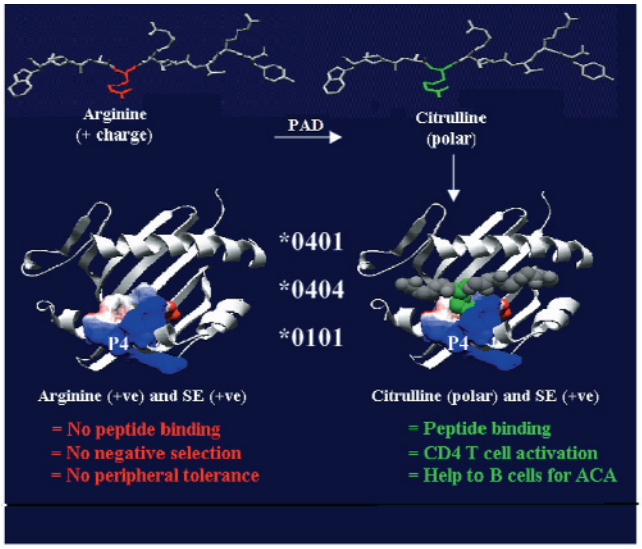
The Joy of Citrulline: New Insights into the Diagnosis, Pathogenesis, and Treatment of Rheumatoid Arthritis

While the cause of rheumatoid arthritis (RA) is not known, it is generally acknowledged to be a chronic autoimmune disorder, and over the past 40 years, beginning with the discovery of rheumatoid factor, evidence has accumulated to support this notion. However, rheumatoid factor is neither specific nor completely sensitive for the diagnosis of RA, and there is no uniform consensus regarding its role in the pathogenesis of the disease. Other self-antigen targets of the immune response in RA have been identified, including collagen II (CII)[1], the most abundant protein of articular cartilage; human cartilage glycoprotein 39 (gp39)[2]; and proteoglycan[3]. While these antigens can induce arthritis in genetically susceptible mouse strains, evidence for their participation in human RA is not clearly defined.

There has been considerable recent interest in the observation that a very high proportion of patients with RA have IgG antibodies to citrullinated peptides. Such anti-citrulline antibodies appear relatively early in RA[4], are highly specific for this disease (98%)[5], and can be measured by quite reproducible, readily available assay systems. While these are relatively recent observations, it is of interest that such disease-specific autoantibodies were first described in RA more than 40 years ago as antiperinuclear factors[6]. A series of studies by several different laboratories have established that the target of these antibodies is filaggrin, a protein expressed in the late stages of terminal differentiation of epithelial cells of mammalian skin and esophagus. It was also established that these autoantibodies target posttranslationally modified or citrullinated filaggrin[7,8].

The posttranslational process of citrullination involves the deimination of arginines in certain polypeptides and is catalyzed by the Ca^{2+} dependent enzyme peptidylarginine deiminase (PAD) (see review[9]). The biochemical outcome of this process is the conversion of positively charged arginines to polar but uncharged citrullines. These changes in the property of citrullinated peptides make them targets of IgG antibodies in RA. This altered property of arginine-containing peptides also allows them to bind with 100-fold higher affinity to the positively charged peptide-anchoring pocket known as P4 in MHC class II molecules expressing the shared epitope (SE) (e.g., HLA-DRB1*0101, 0401, and 0404)[10]. We have recently demonstrated this directly with purified MHC class II molecules expressing the SE and in mice transgenic for HLA-DRB1* 0401 (DR4-IE tg mice). The HLA-DRB1*0402 allele, which is protective for RA, has a negatively charged P4 anchoring pocket that can bind arginine[11] and likely citrulline. This is predicted to result in peptide MHC class II ligands that should negatively select T cells with this specificity in the thymus. T cells with high avidity for this complex should therefore not be expressed in peripheral lymphoid tissues (Figure 1). This may explain why patients with the HLA-DRB1*0402 allele do not appear to develop RA. When DR4-IE tg mice were immunized with certain citrullinated peptides, they produced CD4 Th1 responses thought to be important participants of the immune response in RA. These observations imply that the immune response to citrullinated peptides is influenced by MHC class II genes, which encode the SE. This is consistent with other studies in human RA patients showing a strong correlation between anti-citrulline antibodies and the SE[12]. These experimental observations therefore link 2 commonly observed features of RA: the high frequency of expression of the SE and the common and highly specific occurrence of anti-citrulline antibodies.

One may ask what relevance these findings have to the pathogenesis of RA. Several experimental observations suggest that the immune responses to citrulline could play a significant role in the pathogenesis of RA inflammation. First, the synovial tissue of patients with RA has been shown to contain citrullinated proteins (citrullinated alpha and beta chains of fibrinogen, and citrullinated vimentin)[13,14]. Citrullinated proteins appear to be localized to the cytoplasm of synovial monocyte/macrophage-like cells and interstitial deposits in the deep synovial tissue of RA patients[13,15]. Similarly, in some experimental models of arthritis, citrullinated proteins can be identified in the synovial tissue[16], suggesting that inflammation may upregulate this process, perhaps by increasing the activity of PAD. In an appropriate host (i.e., who expresses the SE), these citrullinated proteins could be targets of the local immune response in the joint. A second observation supporting the role of citrullinated proteins in the pathogenesis of RA



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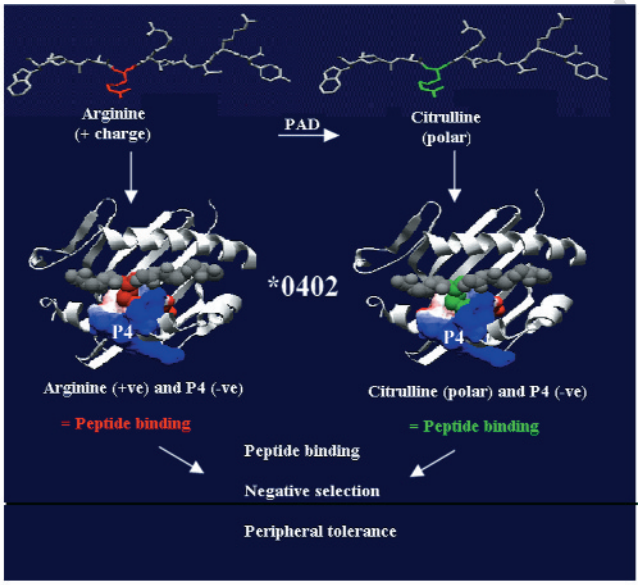

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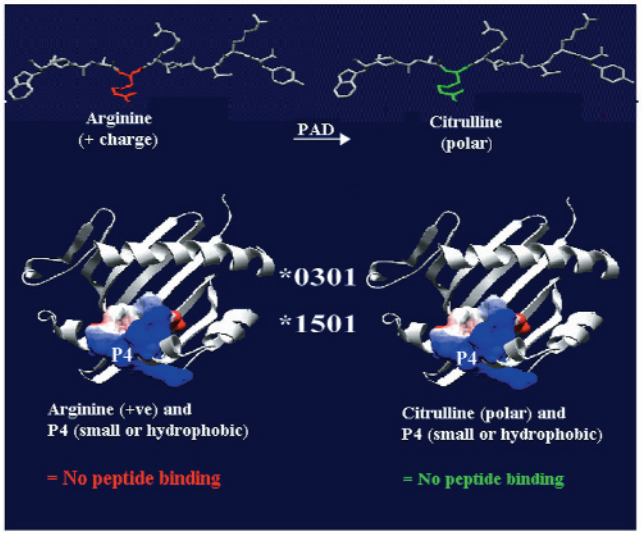

C

Figure 1. The interactions of peptides containing arginine or citrulline with the antigen-binding groove of MHC class II molecules that predispose to RA (A), are disease protective (B), or have no influence on disease (C). A. Normally, endogenous peptides that contain arginine at the position interacting with the P4 anchoring pocket formed by the shared epitope will not bind to these MHC class II molecules due to a clash in positive charge (left side of panel). However, after posttranslational modification by peptidylarginine deiminase (PAD), the positive charge from arginine is replaced with the polar but uncharged citrulline, which can now bind in the P4 pocket (right side of panel). This peptide binding leads to CD4 T cell activation and could provide help to B cells for the production of anti-citrulline antibodies (ACA). B. Disease protective MHC class II molecules contain a negatively charged P4 pocket. This would allow for peptides containing arginine or citrulline at P4 to bind to HLA-DRB1*0402 and be presented to T cells for negative selection and the establishment of peripheral tolerance. C. MHC class II molecules that do not influence disease susceptibility have P4 pockets that are either too small (HLA-DRß1*0301) or too hydrophobic (HLA-DRß1*1501) to accommodate arginine or citrulline, and are therefore unable to bind these peptides.

derives from recent studies in our laboratory[17], indicating that arthritis resembling RA can be induced in mice transgenic for the SE by the administration of citrullinated fibrinogen. Arthritis could not be induced in nontransgenic mice, nor in transgenic mice given noncitrullinated or unmodified fibrinogen.

Finally, it has been recently observed that a cluster of single nucleotide polymorphisms (SNP) on chromosome 1p36, localized at the *PADI4* gene, is associated with RA in the Japanese population[18]. PADI4 mRNA containing the RA associated SNP were more stable than those from the nonsusceptible haplotype. This implies that the mRNA transcript produced from the susceptible PAD haplotype may persist, possibly leading to increased PAD production and more citrullinated protein.

The foregoing observations suggest that genetic factors influencing the expression of PAD could be present in patients with RA. Those RA patients with this PAD haplotype could generate increased quantities of citrullinated peptides, which, in a genetically susceptible host with the MHC class II SE, could lead to the activation of Th cells and IgG anti-citrulline antibodies. Since citrullinated proteins are generated in inflamed synovial tissue[16], these antigens could be targeted by the immune system and further provoke the inflammatory process leading to chronic, persistent synovitis. If immune responses to citrulline play an impor-

tant role in the initiation and perpetuation of RA, then novel forms of therapy targeting these pathways could be developed. One obvious target could be to suppress or block the activity of one or more PAD isoenzymes responsible for producing citrullinated peptides within the synovium. Other approaches could include manipulating the CD4 Th responses that are driving the production of IgG anti-citrulline antibodies. These studies, now being addressed in animal models of arthritis, open a new chapter in our understanding of the pathogenesis of RA and invite the development of new strategies for its management.

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