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Citrullinated proteins in Rheumatoid Arthritis☆

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ABSTRACT

Rheumatoid arthritis is an autoimmune disease of multifactorial etiology characterized by inflammation of the joints and presence of autoantibodies directed against multiple autoantigens. Recently the study of the anti-citrullinated protein antibodies (ACP) has acquired great interest due to its high specificity and sensitivity for diagnosis, in addition to which it has shown to be a predictor of severity in patients with rheumatoid arthritis, suggesting an important participation in the pathogenesis of the disease.

Proteínas citrulinadas en artritis reumatoide

Palabras clave:
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RESUMEN

La artritis reumatoide es una enfermedad autoinmune de etiología multifactorial caracterizada por inflamación de las articulaciones y presencia de múltiples autoanticuerpos. Recientemente, el estudio de los anticuerpos antiproteínas citrulinadas (APS) ha adquirido gran interés debido a su alta especificidad y sensibilidad para el diagnóstico, además de que se ha demostrado que es predictor de severidad en pacientes con artritis reumatoide; lo cual sugiere un papel importante en la patogénesis de la enfermedad.

Introduction

Rheumatoid arthritis (RA) is a widespread auto-immune disease of multi-factorial aetiology and worldwide distribution. Its prevalence is around 1.0% in the adult population and it is more frequent in females than in males (from two to three women for every male affected). The greatest incidence in women occurs between 40 and 60 years of age.[1]

Although it may affect several organs, RA is characterized by the inflammation of the synovial membrane in diarthrodial joints, the vaginae synoviales and sliding synovial bursae. Inflamed synovial tissue presents features of local destruction invading and damaging the joint's structures, resulting in functional loss, giving rise to disability in patients with RA.[2] Affected individuals show a genetic predisposition and HLA-DR1 and DR4 alleles are most often associated with pathogenesis of the disease.[3]

The diagnosis of RA is mainly based on the clinical manifestations following the 1978 classification criteria of the American College of Rheumatology (ACR). It should, however, be pointed out that, the classification criteria include the presence of rheumatoid factor (RF).[4] RF is defined as auto-antibodies that react against the Fc region of IgG isotype immunoglobulins. RF is a non-specific biomarker for RA, as it increases as a general consequence of the activation of the immune response in the context of the formation of immune complexes.[5] In addition, it may be present at high titres in chronic infections and in other auto-immune diseases such as systemic lupus erythematosus (SLE), mixed connective tissue disease (MCTD) and primary Sjögren's syndrome (PSS). It can also be detected in the adult population and in healthy individuals.[6-8]

In recent years, the study of the reactivity of anti-citrullinated protein antibodies has attracted a lot of interest. The antibodies most

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often associated with RA are: anti-perinuclear factor (APF) and anti-keratin antibodies (AKA), both of which target citrullinated fillaggrin[9]; anti-Sa antibodies, which recognize citrullinated vimentin[10] and anti-cyclic citrullinated peptide antibodies (anti-CCP).[6] The latter have a sensitivity in excess of 80% and specificity of 98% in patients with RA.[11] In addition to their high sensitivity and specificity, they present in early stages of the condition.

Peptidyl arginine deiminase and citrullination in RA

Post-translational modifications (PTM) are chemical changes suffered by proteins after being synthesized. One such PTM is citrullination (conversion of residual arginine to citrulline), which is then catalyzed by the peptidyl arginine deiminase enzyme (PAD); 5 isoforms of PAD have been identified as having differential expression in tissues and organs.

The PAD isoforms are widely distributed among the tissues of mammals. PAD1 is predominantly expressed in the epidermis and uterus; PAD2 is the most ubiquitous member of the family and is expressed in skeletal muscle, spleen, brain, salivary glands, uterus, etc.; PAD3 is expressed in follicles; PAD4 is expressed in neutrophils and eosinophils, whereas PAD6 has been detected in ovaries, testes and leukocytes of peripheral blood.

The study of protein citrullination has attracted a lot of interest because its involvement in several physiological and pathological processes. The physiological ones include terminal differentiation of epithelial cells, regulation of gene expression and apoptosis; as for the pathological processes, citrullinated proteins have been linked to disease progression in RA, multiple sclerosis and Alzheimer's disease, among others.

The conversion of arginine into citrulline is capable of activating an immune response. This conversion leads to a change in the amino acid charge. At the protein level, the reaction provokes a reduction of approximately 1.0 Da in the molecular mass for each arginine modified. The positive charge is lost so the isoelectric point (pI) is also altered and the interactions with other proteins may also be affected.[12,13]

In auto-immunity, the expression of PAD4 has been associated with the development of clinical manifestations of RA.[14-17] It has recently been shown that the presence of anti-citrullinated protein antibodies, as well as the expression of PAD4, precedes the onset of clinical manifestations in RA.[14] On the other hand, PAD2 and citrullinated proteins have also been detected in the synovial fluid of patients with RA and spondyloarthritis (SA),[18] suggesting that citrullination is a process associated with inflammation but the generation of pathogenic antibodies recognizing citrullinated proteins is a process specific to RA.[18]

Another important aspect with regard to PAD4 expression is the association between certain polymorphisms and the development of RA. In 2003, Suzuki et al studied single nucleotide polymorphisms (SNP) in a Japanese population and observed an association between functional haplotypes of the *padi4* gene and RA. They identified four *padi4* haplotypes, of which numbers 1 and 2 had a frequency of 82% while numbers 3 and 4 were only 18%. Among patients with RA, 32% presented haplotype 2 compared to 25% among healthy subjects, and this difference was significant (P=.000008).[19] The mechanism through which haplotype 2 of *padi4* can increase susceptibility to RA has not yet been explained. However, the half-life of haplotype 2's mRNA is known to be 11.6 min and that of haplotype 1 is only 2.1 min, so susceptibility may be explained by the high possibility of PAD translation, resulting in a larger amount of enzyme and consequently increased citrullination of proteins (fibrinogen or vimentin). This might stimulate both the innate and the adaptative immune response, thus allowing the development of chronic inflammation.[19] Suzuki's study has aroused great interest even though the phenomenon was not corroborated in RA-affected populations in France, United Kingdom and Spain.[20-22] Only one study conducted in a Korean population confirmed the association,[23] suggesting a major participation of polymorphism in the Asian population but not in European Caucasian populations.

Citrullinated proteins in RA

In 1964, Nijenhuis and Mandema first described APF antibodies in patients with RA.[24] In 1979, Young showed that the sera of patients with RA reacted against the oesophageal epithelium of rats and defined these antibodies as AKA antibodies.[25] Both auto-antibodies, detected by means of indirect immunofluorescence techniques, showed a high degree of specificity in RA (approximately 94%). However, due to their limited sensitivity (40%-55%), the technical difficulties involved in their determination and the absence of standardization in the techniques applied, studying AFP and AKA auto-antibodies was the exclusive domain of researchers and specialist immunology laboratories.

In 1995, Sebbag et al showed that both AKA and AFP antibodies recognize molecules related to fillagrin and profillagrin.[26] They subsequently noted that the sera of patients with RA presented greater reactivity against in vitro profillagrin.[26] Nonetheless, in later studies using recombinant fillagrin or fragments of synthetic profillagrin peptides, the sera of patients with RA did not show any reactivity.[27] The foregoing suggested that the immunogenicity of fillagrin and profillagrin was related to PTM. In the same paper, Girbal-Neuhaser showed that the antigen recognized by the AKA and AFP antibodies was citrullinated profillagrin.[27] Notwithstanding, a detailed study revealed that there is no *in vivo* expression of profillagrin in synovial tissues.[28] This excluded the possibility that the antigen recognized *in vivo* by AKA and AFP could be profillagrin, as inflammation only occurs in the joints and not in the epidermis, where profillagrin is expressed more abundantly.[28] Later studies showed that both the α and β chains of the citrullinated fibrin are the antigens recognized by APC antibodies and are present in patients with RA.[11]

Due to the importance of detecting APCs, there have been several studies related to the identification of these or the dominant citrullinated antigens. Various citrullinated proteins have been described as having high specificity for RA, including type I and type II collagens (CI and CII),[29,30] fibrinogen,[28] and vimentin.[10,31] Matsuo et al analyzed the proteomic profile for the synovial tissue of a patient with RA and detected 51 citrullinated proteins, of which 30 (58.8%) were antigenic.[32] Thirteen of the 30 proteins were identified as derivatives of fibrinogen, asporin and the α sub-unit of the actin-F capping protein (CapZα-1). In addition, they detected antibodies against CapZα-1 in 16 of the 30 sera of patients with RA (53.3%), in 2 of the 28 patients with osteoarthritis (7.1%) and in 2 of the 31 healthy individuals (6.5%).[32] Another important antigen, reported to be the target for APCs, is citrullinated enolase-α, identified by means of immunohistochemistry in slices of synovial tissue from patients with RA. In 2005, Kinloch et al reported the presence of antibodies against enolase-α in 46% of the sera from patients with RA.[33]

The published data show the existence of multiple proteins targeted by APCs, all presenting different levels of sensitivity and specificity for the diagnosis of RA. In a study conducted at our laboratory, we identified epitopes of enolase-α sharing homology with residues adjoining citrulline in sequences of CII, fibrin and vimentin; this might explain the similarity in the specificity of the antibodies recognized by these proteins and present in patients with RA.[34]

Pathogenic role of citrullination in RA

Recent papers on models of CII-induced arthritis show the participation of citrullination in the auto-immune response. In

the Lew.1AV rat model, Lundberg et al showed that citrullination of collagen is a powerful mechanism to increase self-reactivity and that the APC antibodies present crossover reactivity against both citrullinated and native CII. In addition, they proved that the severity of the arthritis correlates with PAD4 expression in the infiltrate of mononucleate cells and with the amount of CII citrullinated.[35] In another study, Hill et al showed that transgenic mice engineered for the molecule in the main DRB1*0401 histocompatibility complex and immunized with human citrullinated fibrinogen developed progressive arthritis in the presence of APCs.[36,37]

On the other hand, one of the factors related to the increase in the risk of developing RA is smoking. In 2006, Klareskog et al observed a correlation between the presence of the HLA-DRB1*0401 allele with APC antibodies in smoking individuals with RA. The relative risk of developing RA and the presence of positive APC antibodies is 20 times greater for patients who smoke and have the HLA-DB1*0401 allele than for non-smokers without the allele.[38]

The association between the citrullination of proteins in smokers' lungs and the start of the immune response against these proteins in RA is a phenomenon that might not be exclusive to smokers.[39] Exposure to other pollutants may cause harm to lung tissue, release of PAD, which boosts the citrullination of proteins released by the damage and, in genetically susceptible individuals, increases the risk of developing auto-immunity.[40]

Conclusions

- The presence of auto-antibodies recognizing citrullinated proteins is a specific serological marker for RA.
- The PAD2 and PAD4 isoforms are the enzymes associated with the generation of citrullinated auto-antigens in RA.
- Citrullinated auto-antigens showing the greatest specificity for RA are: fibrinogen, vimentin, CII and enolase-α.
- The presence of citrullinated proteins and genetic predisposition are two important factors associated with the development of arthritis.
- There are other factors such as tobacco consumption that are involved in the onset of RA.

Conflict of interest

The authors state that there is no conflict of interest.

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