Editorial

Citrullinated Antigens: Just Diagnostic Tools or Pathogenic Targets in Rheumatoid Arthritis?

The diagnostic procedures of rheumatoid arthritis (RA), being the former domain of clinical and imaging findings, were significantly improved with the introduction of novel immunoassays. Thus, autoantibodies against citrullinated antigens were confirmed by numerous studies to represent powerful markers for established RA, and moreover were found to be highly specific for early and undifferentiated disease manifestations[1-3]. Moreover, identification of citrullination as an important posttranslational modification of autoantigens in RA has not only provided a valuable diagnostic tool, but, surprisingly, has also opened a door for several old, as well as novel, promising antigens. However, the pivotal question remains: Is this only an epiphenomenon of the complex autoimmune disorder RA, or a part of the pathogenesis?

Citrullination is a posttranslational modification of arginine residues catalyzed by a family of calcium-binding enzymes, the peptidylarginine deiminases, of which 5 different but strongly related isoforms have been identified to date[4]. The product of citrullination is the non-standard amino acid citrulline, which contributes to the backbone of certain proteins. By decreasing the net positive charge, citrullination can alter the primary, secondary, and tertiary structure of a protein, with potential influence on intermolecular interactions.

Such a modification of proteins is involved in several physiological processes, e.g., conversion of filaggrin in keratinocyte differentiation or of vimentin in apoptosis[5,6]. Interestingly, distinct haplotypes of the peptidylarginine deiminase 4 gene were found to be related to the manifestation of RA in Asian but not European populations, and the enzyme itself was identified as an autoantigen[7-10].

It was shown that citrullination occurs within the inflamed synovial tissue[11]. However, filaggrin as well as the synthetic antigen cyclic citrullinated peptide (CCP) are not expressed in the synovium, and therefore are unlikely to represent the primary targets of the autoimmune response. Subsequently, citrullinated fibrin, as a cleavage product of fibrinogen, was identified as the major target of antibodies against citrullinated antigens in RA, exhibiting a close cross-reactivity to filiggrin[12,13]. Fibrinogen is one of the predominantly citrullinated proteins found in synovial tissue; it was also detectable as a circulating antigen in synovial fluid of patients with RA[14]. Interestingly, it was also shown that synovial exosomes contain citrullinated proteins including fibrin derived molecules that could play a role in the induction and distribution process of citrullinated autoantigens[15].

In this issue of *The Journal*, Hill, *et al* provide further important evidence that citrullinated fibrinogen represents a major autoantigen in established RA[16]; moreover, their results confirmed a high disease specificity for this antibody response. By using *in vitro* citrullinated fibrinogen as an antigen in ELISA, the sensitivity of the assay was 75%, with a specificity of 98% for RA, making it comparable to anti-CCP. Although the cohort of patients with RA analyzed was relatively small, appropriate control patients with other rheumatic disorders were included. Importantly, the antibody reactivities against citrullinated fibrinogen were consistent with the anti-CCP results, with the exception of only one patient.

The results of Hill, *et al* are in good agreement with a recently published study revealing a similar diagnostic performance for anti-citrullinated fibrinogen antibodies and the second generation of CCP-ELISA[17]. Citrullinated fibrinogen was also shown to provide a similar sensitivity and specificity compared to CCP in patients with early RA; moreover, both markers were good predictors of radiographic progression[18]. Another study revealed an association between the HLA-DRB1*0404 genotype and the generation of anti-citrullinated fibrinogen antibodies. Further, a frequent T cell response was observed against fibrinogen in RA[19].

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Citrullinated fibrinogen is immunogenic, but the antibody response is not necessarily accompanied by an arthritogenic phenotype in rodents[20,21]. However, it was recently shown that induction of tolerance to a citrullinated peptide led to a significant reduction in susceptibility to collagen induced arthritis in mice[22]. Further, administration of monoclonal antibodies against citrullinated fibrinogen was able to overcome the protective tolerance to substantially enhance arthritis. By demonstrating pathogenicity in an animal model, these encouraging results clearly support the link between altered antigenic properties due to citrullination of fibrinogen and the manifestation of arthritis.

However, many questions remain. First, in order to establish anti-citrullinated fibrinogen antibodies as a diagnostic marker, standardized assays must be introduced. Subsequently, it will be necessary to conduct multicenter studies in larger cohorts including early and undifferentiated disease manifestations, as well as comprehensive followup analyses, in order to clarify the significance of these antibodies in correlation to disease activity and outcome.

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