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Review

Biomarkers for rheumatoid arthritis: From molecular processes to diagnostic applications-current concepts and future perspectives

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ABSTRACT

Early diagnosis and immediately started appropriate treatment are mandatory for the prevention of radiographic progression, functional disability and unfavourable disease outcome in rheumatoid arthritis (RA). The current classification criteria for RA include two different types of biomarkers representing inflammatory processes, such as C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) or immune processes including autoantibodies, such as rheumatoid factor (RF) and antibodies against citrullinated proteins (ACPA). After the discovery of RF, the recent recognition of various autoantibodies against post-translationally modified proteins opened new avenues to diagnosing RA and predicting the course of the disease. Citrullination and carbamylation of amino acids generate new epitopes that can potentially promote the production of novel autoantibodies. In spite of growing knowledge, the pathogenic role of these autoantibodies is still not fully elucidated in RA. In this paper, we review the currently available and novel promising immune biomarkers, which may help in early diagnosis and estimating prognosis in RA.

1. Introduction

Rheumatoid arthritis (RA) is a common systemic autoimmune disease characterized by synovial inflammation, which potentially leads to debilitating and irreversible joint destruction. A combination of multiple factors, such as genetic, environmental and hormonal factors, leads to the development of certain autoimmune processes and subsequent tissue damages characteristic to the disease [1]. Based on our present knowledge, there are at least two distinct RA subsets. The most common type is associated with certain HLA DRB1 haplotypes, production of antibodies against citrullinated proteins (ACPA), and has a more aggressive disease course [2–4]. Current diagnostic criteria rely on clinical findings and the following laboratory tests: acute phase reactants, rheumatoid factor (RF) and ACPA. Equal weight is given to RF and ACPA [5]. Early diagnosis and immediate, effective therapy are crucial for the prevention of joint deterioration, functional disability and unfavourable, even fatal disease outcome. The current, optimal management of RA requires that adequate therapy should be initiated within 3–6 months after the onset of the disease. Therefore, to achieve remission, there is a narrow “window of opportunity” [6–8]. In this review, we discuss the currently available and novel promising autoimmune biomarkers for RA, which may help in early diagnosis and estimating prognosis.

2. Rheumatoid factor

The discovery of RF, first reported in 1940, was a breakthrough in the serological diagnostics of RA [9]. RF, which is an autoantibody reacting against the Fc portion of IgG antibodies, occurs in about 70% of patients with established RA, less frequently in early RA [10]. Nevertheless, RF may be positive many years before the onset of disease, thus its presence may also indicate an increased risk of disease development [11]. RF is one of the most widely used biomarkers in establishing RA diagnosis [12], even though negative result does not rule out RA, and positive results can be due to other diseases. RF also occurs in several other systemic autoimmune diseases, such as Sjögren’s syndrome (75–95%), mixed cryoglobulinemia (monoclonal RF, 100%), systemic lupus erythematosus (SLE) (15–35%), mixed connective tissue disease (MCTD) (50–60%) and primary biliary cirrhosis (45–70%). Increased RF levels may be also detected in various infectious diseases, e.g. hepatitis C (40–75%), Epstein-Barr virus infection (20%), cytomegalovirus infection (20%) and subacute bacterial endocarditis (40%)

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[13–16], as well as in many inflammatory conditions and malignancies [17]. The frequent occurrence of RF in other conditions limits its specificity in RA; moreover, approximately one third of patients with early RA do not have RF; although, later a part of them may became positive [18]. Based on systematic reviews and meta-analysis of the literature, the sensitivity of RF is only 60–75% with a specificity of 40–85%; furthermore, results from studies of patients with early rheumatoid arthritis were similar to those from all studies [19–21]. RF does not generally help in monitoring the disease [13], although it may help with the use of certain biologics, such as etanercept and infliximab, when levels of rheumatoid factor may decrease along with the clinical disease activity [22,23].

3. Autoantibodies against citrullinated proteins (ACPA)

Before the importance of citrullinated protein epitopes in RA was discovered, three autoantibodies were known already, which later were found to be members of the ACPA family. These were anti-perinuclear factor (APF), anti-keratin antibodies (AKA), and anti-Sa. APF, described first in 1964, targets keratohyalin granules around the nuclei of buccal mucosal epithelial cells [24]. AKA, described in 1979, reacts with stratum corneum epithelial cells of rat oesophagus [25]. The specificities of both markers are similar (90% or higher), and the sensitivities are also in the same range (35–70%) [26,27]. Both autoantibodies may serve as early diagnostic markers, since they are detectable before classical clinical symptoms appear [28,29]. However, only AKA was found to have convincing evidence of a prognostic value in undifferentiated peripheral inflammatory arthritis [12]. In 1995, it was shown that AKA and APF largely recognize the same autoantigens, filaggrin (filament-aggregating protein) or pro-filaggrin [30], and three years later, it was revealed that the target epitopes were all citrullinated [31,32].

Anti-Sa is an RA-specific autoantibody discovered in 1994, which recognized a 50 kDa protein in immunoblots of spleen and placenta extracts. This autoantibody was shown to be present in the sera of 43% of RA patients but rarely in patients suffering from other autoimmune diseases or in healthy individuals. In addition, 27% of RF negative RA patients were positive for anti-Sa [33]. Several studies addressed the diagnostic sensitivity and specificity of anti-Sa, and found a specificity of 92–100%, with a sensitivity of 32–43% [34]. The high specificity was coupled with substantial prognostic value, as anti-Sa positivity has been associated with more active and destructive disease [35,36]. Thus, anti-Sa has been thought to have important diagnostic and prognostic relevance in RA. A decade after its discovery, anti-Sa was shown to target citrullinated vimentin [37]. Since then, numerous other citrullinated autoantigens have been identified, including fibronectin, filaggrin, fibrinogen, vimentin, and collagen [38].

3.1. Citrullination of proteins in RA

Since citrulline cannot be incorporated into *de novo* synthetized polypeptides (there is no citrulline transfer RNA), citrullination can happen during the post-translational modification of arginine-containing proteins in which arginine side chains are hydrolyzed, resulting in the replacement of one of arginine's terminal nitrogen atoms with a doubly bonded oxygen atom (deimination). Positively charged arginine side chains are thereby converted to neutral citrulline side chains. The modification of proteins can generate new epitopes, different from those to which the immune system is tolerant, thus leading to a possible immune reaction against citrullinated antigens. Additionally, these small modifications can further enhance the immunogenicity of the proteins due to an increased uptake and presentation of antigen presenting cells [39]. Citrullination is catalyzed by Ca^{2+} dependent enzymes, denoted as peptidylarginine deiminases (PADs). Five isoforms of PAD exist, namely PAD1, 2, 3, 4 and 6. These have different tissue distribution, and may also preferentially citrullinate different proteins [40,41]. The PADs have numerous physiological functions; citrullination plays important roles especially during differentiation and development. PAD4 contributes to gene regulation by citrullinating histones [42], and importantly, this isoform is responsible for the generation of new autoantigens in RA. Since the enzyme is not expressed in the thymus, T cells reactive to citrullinated antigens could be not eliminated, which leads to a potential immune reaction against citrullinated proteins [39]. Of note, citrullination play an important role in cell death processes as well, such as apoptosis, autophagy and neutrophil extracellular traps (NET) formation, which are characterized by higher levels of calcium, thus leading to subsequent PAD activation. During the apoptosis, vimentin is citrullinated, which is needed to prepare intracellular proteins for degradation [43]. Citrullination of histones by PAD4 is essential in the massive decondensation of chromatin that occurs in NET formation [44]. In RA, NETs represent an important source of autoantigens bearing posttranslational modifications and fueling the production of ACPA promoting pathological immune responses and derailed inflammation [45,46].

Interestingly, PAD was found in Porphyromonas gingivalis (PPAD), which is a major pathogen bacterium in periodontitis (PD). PPAD is active at higher pH levels, it is not dependent on Ca^{2+}, and preferentially citrullinates C-terminal arginines, both peptide-bound and free ones, while human PADs do not [47]. The chronic exposure to the citrullinated peptides generated by P. gingivalis at periodontal sites may contribute to the breakdown of immune tolerance and trigger an immunological response to citrullinated proteins in a subset of RA patients with PD, who have shared epitope. A recent study reported that PD patients are exposed to citrullinated histone H3 in inflamed periodontal tissue; and citrullinated histone H3 is targeted by autoantibodies present in RA sera. These findings confirm the role of periodontitis in the generation of new antigens targeted by autoantibodies in RA [48].

Recently, it has been revealed that PAD4 can undergo autocitrullination, which might be a regulative process that inactivates the same enzyme; however, in the other hand it modifies the structure of the enzyme, which probably increases its recognition by human autoantibodies [49]. This is in line with the observation that autoantibodies targeting PAD4 seem to have predictive and prognostic relevance in RA patients [50]. Interestingly, PPAD can be autocitrullinated as well and may perpetuate the immune response through epitope spreading and cross-reactivity with citrullinated human proteins. However, its significance is controversial since it was reported that anti-PPAD antibodies do not correlate with ACPA levels and disease activity in RA, moreover it may even play a protective role for the development of PD in RA patients [51].

The presence of several citrullinated proteins has been already demonstrated in the RA synovial tissue and fluid [52–54]. Synovial lymphocytes and macrophages express PAD2 and 4 with expression levels that correlate with the intensity of inflammation. The enzymes are found within, or in close vicinity to fibrin deposits [55]. Synovial fibroblasts respond to fibrin deposits by producing pro-inflammatory cytokines, and the pro-inflammatory response is enhanced when the fibrin is citrullinated [56], indicating that citrullinated peptides play a central role in these autoimmune processes. Previously, ACPA-secreting intra-synovial B-cells have been isolated from RA, emphasizing the importance of these antibodies in the pathogenesis of the disease [57]. ACPA autoantibodies lead to immune-complex formation and synovial inflammation, subsequent monocyte, and granulocyte activation, as well as pro-inflammatory cytokine and chemokine secretion.

3.2. Clinical relevance of ACPAs

The determination of ACPAs may have important prognostic significance, since ACPA production can precede the onset of clinical RA symptoms by several years [58,59]. ACPA-positive individuals with early, undifferentiated arthritis have higher risk to develop subse-