Anti-Cyclic Citrullinated Peptides
Autoimmunity in the Pathophysiology and Diagnosis of Rheumatoid Arthritis

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Abstract—The discovery of Anti-Cyclic Citrullinated Protein (CCP) more than 20 years ago gave rise to the investigation of RA pathophysiology. The study of these autoantibodies illuminated autoimmune pathways involved in RA pathogenesis and clarified some aspects of the role of other molecular systems (MHC class II) in the development of RA. On the clinical field, they have proven to be reliable markers for the diagnosis and assessment of prognosis of RA. Anti-CCPs revolutionized the way we perceive RA pathophysiology and have recently been implicated in the diagnosis of RA.

I. INTRODUCTION

RHEUMATOID arthritis is the most common autoimmune inflammatory disease of connective tissue, involving 1-2% of the general population worldwide. During the past decades, autoimmune disorders have been correlated with several autoantibodies. Despite the increased incidence in respect to other autoimmune diseases, the pathophysiology and evolution of rheumatoid arthritis have not been strongly associated with the presence of specific antigens and autoantibodies.

For many years, the study of RA pathophysiology revealed several antigens that might be involved in the pathogenesis of the disease. These antigens were classified into two major categories. Synovium-specific autoantigens, and autoantigens not associated to the synovium [1]. The first category includes type II collagen, proteoglycans and other molecules of connective tissue. The second group includes i) foreign antigens, such as heat shock proteins (HSP) which induce autoimmunity by the mechanisms of molecular mimicry, ii) common autoantigens, including BiP, glucose-6-phosphate isomerase, iii) post-translationally altered proteins, such as autologous IgG immunoglobulin (which is the target molecule of rheumatoid factor - RF) and CCP. These last proteins have been implicated in the pathophysiology of RA and are responsible for the induction of specific autoantibodies, the anti-CCPs.

Until recently, RF was the autoantibody that correlated most with the diagnosis and prognosis of RA. Nevertheless, high prevalence of this autoantibody in other autoimmune or infectious systemic diseases (Sjogren syndrome, bacterial or viral infections, liver cirrosis, etc) implies that the assessment of rheumatoid factor in the diagnosis of RA lacks of high specificity. On the contrary, accumulative clinical data showed that anti-CCPs correlate more specifically to the diagnosis of RA than RFs and predispose to a severe pattern of disease indicating poor prognosis.

Initial studies of ACPA (anti-citrullinated protein antibodies) were published in 1964, when anti-perinuclear factors (APF) were described in the serum of RA patients [2]. Further studies, in 1979, revealed the presence of anti-keratin autoantibodies (AKA) [3]. These autoantibodies were correlated to RA more than any other serological index, but found no major application since their target molecules had not yet been described. It took up to two decades to identify that the target molecules of these autoantibodies were citrullinated proteins [4].

The process of Citrullination

The introduction of anti-CCPs caused increased interest for the description of the biochemical pathways that are responsible for protein citrullination. Citrullination is a process by which arginine is transformed into citrulline. Citrulline is an aminoacid for which mRNA has not been found, indicating that it originates as a result of post-translational protein modification. During citrullination, an amminogroup of the lateral chain of arginine is replaced by singlet oxygen (O) with the release of H+ and ammonia. This reaction is catalysed by peptidylarginine deiminase (PAD) and results in altering the electrical cargo of the protein, since a positively charged aminoacid (arginine) is replaced by an aminoacid with neutral cargo (citrulline). The process results in the reduction of the positive charge, alters the binding compound of the protein and eventually leads to modification of its geometrical architecture. As a result, citrullinated protein unfolds, revealing inner structures and potentially inner cryptic antigenic epitopes. Furthermore, the inner structures revealed are exposed to enzymatic processes that may lead to the production of neo-antigens. These modifications are implied to be responsible for the development of autoantigen targets in the...
synovium (citrullinated proteins) that contribute to the pathogenesis of RA [5].

The role of Peptidylarginine deiminase

PAD enzymes are a group of proteins that catalyze the process of citrullination. These enzymes have been studied both in human and animal models and PAD activity has been found even in monocellular microorganisms. These enzymes use Ca++ as coenzyme (concentrations of Ca++>10^-5mmol/L are required). Up to date, 5 human isoforms of this enzyme have been described that differ mainly for their distribution to several tissues. PAD 1 was described in the epidermis and in the uterus. Its substrate is fillagrin, a protein associated to the production of intermediate filaments. PAD 2 is the most common isoform, and has been isolated in skeletal muscles, brain, spleen, various glands etc. It is responsible for the citrullination of myelin basic protein MBP and vimentin, structural component of intermediate filaments that is found in mesenchymal cells and in monocytes-macrophages. PAD 3 is found in the hair calices of the epidermis and induces conformational changes in the proteins of the hair. PAD 4 was first described in mice. The analogous human isoform is PAD 5. Both PAD 4 and PAD 5 are referred today as PAD 4. This isoform is found in neutrophils and monocytes, and in contrast to all other isoforms that reside to the cytoplasm, this isoform is found in the nucleus and has nucleoproteins as a substrate. Recently a PAD 6 isoform has been described in the foetus, citrullinating intermediate filaments of the cytosol, indicating potential role in the development of embryogenesis. The genes coding for all these isoforms are located in chromosome 1, on locus p.36.1 [5].

From all above isoforms, PAD 2 and PAD 4 are involved in the pathophysiology of RA.

Citrullinated proteins involved in RA pathophysiology

The process of citrullination executed by PAD2 and PAD4 enzymes is a non-specific, post-translational process that involves miscellaneous proteins in various tissues.

The high association of anti-CCP autoantibodies with RA raised a persistent scientific research in order to identify the specific group of citrullinated proteins that are involved in RA pathophysiology.

It was proved that citrullination of functional collagen II is responsible for anti-CCPs induction. Nevertheless, other citrullinated proteins have been described in the pathophysiology of RA (a-enolase, vimentin, collagen type I and II telopeptides) and the family of anti-citrullinated protein antibodies (ACPA) is expected to grow [6]. Immunoenzymatic assays have been developed for some of these autoantibodies (anti-CCPs -1, -2, -3, anti-MCV, etc) focusing on different citrullinated proteins from the group of ACPA. The first generation assays for anti-CCPs detection was replaced by a second generation assay, which proved to be of higher sensitivity (88% versus 53% respectively), even though both methods shared almost same specificity (95% versus 96%) [7, 8]. A third generation of anti-CCPs assay has been developed but its application did not add any further qualitative characteristics to the widely used anti-CCPs2 assay [9].

The role of anti-CCPs in RA pathophysiology

Citrullination is a ubiquitous phenomenon that takes place in various tissues and stages of cellular differentiation. It is a marker of high cellular differentiation and apoptosis. Keratin cells of the epidermis normally express a high proportion of citrullinated peptides, whereas nucleoprotein citrullination is a marker of degeneration of cellular DNA. This overgrowing study of anti-CCPs posed a new question to researchers: why only RA patients specifically develop autoimmune mechanisms against these proteins, since citrullination is a common process in cell biology. In order to answer this question considerations regarding genetic polymorphisms and environmental parameters were proposed [10].

Initially it was thought that in RA, a genetic predisposition for augmented citrullination is implicated in the development of autoimmunity. This speculation was confirmed by animal models with the discovery of single nucleotide polymorphisms (SNPs) on PAD 4. 17 SNPs have been described 17 SNPs, two of them correlating strongly with RA [11]. It is believed that SNPs correlating to RA provide a more stable isoform of mRNA that favours increased transcription and production of PAD enzymes. Furthermore, these SNPs provide PAD enzymes with i) capability of activation in decreased Ca++ concentrations ii) proper intracellular topography and iii) increased substrate specificity, rending these PAD isoforms more efficient thus favoring the process of citrullination [12].

Despite the detection of genetic predisposition to citrullination, another question remains to be answered: why anti-CCP autoantibodies are RA specific, considering that augmented citrullination occurs in osteoarthritis or reactive arthritis models. Once again, it was proposed that there is a disturbance in the humoral reactivity against CCPs in RA, which differentiates this process from the citrullination occurring in other inflammatory reactions. It was described indeed that mice with inflammatory arthritis had increased levels of CCPs in the synovium but no anti-CCPs were detected in the peripheral blood [13], indicating that corruption of humoral tolerance against CCPs is RA depended.

All above data imply that citrullination is a pathogenic process involved in the development of RA: any disturbance of tissue homeostasis (infection, trauma) leads to inflammation, creating micro environmental changes in the synovium that predispose to oxidative stress. As a result, activated monocytes and granulocytes infiltrate the synovium. Their degeneration releases intracellular PAD enzymes in the extracellular matrix. In this new environment, Ca++ concentrations are high enough to activate PAD enzymes and the process of citrullination begins. On the other hand, humoral activity leads to anti-CCPs production, and the immunocomplexes derived maintain chronic inflammation in the synovium. This circuit of citrullination and immunocomplex formation favors favours the development of chronic inflammation in the synovium and contributes to the progression of joint degeneration [14]. On this regard, many theories implicating environmental factors and smoking in the pathogenesis of RA through CCPs production have been proposed [15]. It is our belief that all these environmental factors act in the pathogenesis of RA only.
by enhancing oxidative stress and CCPs production. Once a subject is exposed in these environmental parameters, the development of RA is a condition that depends mainly on individual predisposition, expressed by genetic factors described above.

The role of MHCII in CCPs recognition

It is well known that the presence of the shared epitope predisposes for to severe RA. It has been described that the MHC molecules involved in anti-CCPs production are the same that predispose to the development of RA.

Citrullinated proteins have a high affinity for the recognition site of MHCII molecules containing the shared epitope (P4) [16]. This P4 site of MHCII has greater affinity for neutral or negatively charged amino acids due to its tridimensional conformation. Citrullination on the other hand, diminishes the positive charge of proteins rending them more compatible for interaction with P4. The CCPs-MHCII interaction initiates a clonotypical T cell expansion of T-helper 1 type [17]. The induction of a Th1 response is supported by the finding that the IgGs produced against CCPs are mostly IgG1 and less IgG4, characteristic pattern of Th1 responses [18].

All above observations, apart from illuminating the role of CCPs in the pathophysiology of RA, clarify the role of the shared epitope in the development of the disease. Citrullination multiplies up to 100 times the affinity of CCPs for MHCII. The increased interaction of CCPs-MHCII on antigen presenting cells overlaps the safety cut-off of T-cell anergy and leads to clonotypical T cell expansion. MHCII molecules not associated with RA (e.g. *0301) do not interact vigorously with CCPs due to their P4 conformation and electrical status. Other MHCII molecules, characterized as protective against the development of RA (e.g. *0402), may be reacting with CCPs and are thought to induce negative selection mechanisms or expansion of suppressor CD4+CD25+ regulatory T cells that promote diverse mechanisms of immune tolerance [17].

The role of anti-CCPs in the diagnosis and RA prognosis

Sensitivity of anti-CCPS in the diagnosis of RA is about 80% (equal to that of RFs), whereas specificity rises up to 99% [19]. While RFs are also found in other autoimmune inflammatory conditions, the presence of anti-CCPs is high specific for RA. Retrospective studies showed that 50% of RA patients were anti-CCPs positive even 14 years before the clinical onset of the disease [20]. Several studies indicate that the specificity obtained with the co-evaluation of RFs and anti-CCPs is equal to that obtained when anti-CCPs are evaluated alone [21]. Furthermore, co-evaluation of RFs and anti-CCPs results in diminished sensitivity, since it is less probable for a RA patient to be both RF and anti-CCP positive. This observation must be taken into consideration when patients with undifferentiated arthritis are evaluated [16].

Anti-CCPs predispose for severe, erosive RA with poor prognosis [22]. Their presence, as the presence of RFs, is associated with increased incidence of extra-articular manifestations [16]. When both anti-CCPs and shared epitope are found, a very erosive form of RA is expected. Data from meta-analysis studies showed that when the shared epitope is co-

estimated with the presence of anti-CCPs and not with the presence of RFs, then differential diagnosis is more precise for recognition of RA in patients with undifferentiated arthritis. In these patients, the presence of anti-CCPs increases the probability of later onset of RA by 16.7 times. In young RA the presence of anti-CCPs once again predisposes for severe disease [23].

It has recently been described that in early stages of RA, levels of anti-CCPs may initially decline, but soon they recover up to original levels. Therefore, anti-CCPs levels cannot be used as a follow up index in RA patients, and their assessment is useful for estimating only disease diagnosis and prognosis.

Anti-CCPs and biologics

The era of anti-TNF blockade provided substantial benefit in RA evolution. The use of biological agents was found to induce an initial decline on anti-CCPs levels [24, 25]. This decline though temporary, is not associated with clinical remission induced by these therapies [26]. This observation does not allow for the moment the direct association of anti-CCPs levels with RA activity (in contrast to RFs) [27]. Further investigation will estimate the effects of anti-TNFa treatment in the production of anti-CCPs. As for the other biological DMARDs (anti-CD20, anti-CTLA4, anti-cytokine targeted therapies) collective date is expected to illuminate both cytokine network and anti-CCPs production in terms of pathological process [28].

Anti-CCPs and RA diagnostic criteria

Generally, a serological index must fulfill certain qualitative characteristics in order to be adopted as a marker for a disease. These characteristics include 1. High sensitivity and specificity for the disease. 2. Detection in the very early stages of the disease. 3. Implication in disease prognosis. Anti-CCPs satisfy all above conditions since they are more specific for RA than RFs, they are detectable even 14 years before the onset of the disease and they predispose to erosive RA. In 1987, ACR defined 7 criteria for the diagnosis of RA. The presence of RFs is among them. As up to date, anti-CCPs have proven to be more valuable in the overall estimation of RA than RFs. On 2010, a collaborating ACR-EULAR study group on RA introduced adopted the use of anti-CCPs in the diagnosis of RA and its qualitative assessment in terms of severity. Many years after their initial discovery, and after having elucidated pathophysiology aspects of RA, anti-CCPs are finally evaluated for the diagnosis and prognosis of RA [29].

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REFERENCES


