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B-cell epitopes of the intracellular autoantigens Ro/SSA and La/SSB: Tools to study the regulation of the autoimmune response

John G. Routsias, Athanasios G. Tzioufas*

Department of Pathophysiology, School of Medicine, University of Athens, 75 M Asias st, 11527 Athens, Greece

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A common serologic finding in systemic autoimmune diseases is the presence of autoantibodies against intracellular autoantigens. Although their pathogenesis is not fully understood, autoantibodies are important tools for establishing diagnosis, classification and prognosis of autoimmune diseases. In Systemic Lupus Erythematosus (SLE) and Sjögren's syndrome (SS) autoantibodies mainly target multicomponent ribonucleoprotein complex Ro/La RNP. The last years, the main characteristics, the clinical significance of the anti-Ro/SSA and anti-La/SSB autoantibodies, their biologic function, as well as their B-cell antigenic determinants (epitopes) have been addressed. More specifically, the structural characteristics and clinical associations of epitopes along with their utility as tools to investigate the autoimmune response have been investigated in detail. New insights for the pathogenetic role of epitopes in initiation, propagation and regulation of systemic autoimmunity have been emerged. In this regard, the role of epitope spreading in the diversification of autoimmune response and the anti-idiotypic antibodies in the regulation of autoantibodies (idiotypic) response are addressed.

1. Introduction

In 1991, our laboratory prompted by professor Harry Moutsopoulos, focused on the detection of fine specificity of autoantibodies of intracellular autoantigens. At that time most of the autoantigens had been recently cloned and eventually, their primary structure had been resolved. The purpose of such a study was (a) to understand the structures, within the autoantigen, recognized by autoantibodies and investigate if they share in common sequences with foreign autoantigens, (b) to investigate whether certain epitopes are associated with different diseases, disease subtypes and individual manifestations or even its activity and severity and (c) to develop methods for autoantibody detection, characterized by high sensitivity and specificity.

After several years of intense research, ours and others laboratories realized that the detection and study of B-cell epitopes gave us important insights on the mechanisms involved in the perpetuation and regulation of the autoimmune response. In this review the major advances of B-cell epitopes of intracellular autoantigen, particularly those directed against Ro/SSA and La/SSB are discussed.

2. Major intracellular autoantigens-rationale for the detection of epitopes

Sera of patients with systemic autoimmune diseases often contain autoantibodies directed against intracellular complexes composed of a number of proteins that are non-covalently associated with nucleic acid components [1]. Many of these autoantibodies are essential for the clinical evaluation of patients with systemic rheumatic diseases, since: (i) they are included in the diagnostic or classification criteria of certain systemic autoimmune disorders [2,3], (ii) they are associated with disease activity indices, particularly in SLE [4] and (iii) some of them might be correlated with specific clinical manifestations in the spectrum of a given systemic disease [5]. In basic research, the most intriguing question is why these particular autoantigens, among thousands of molecules expressed in the organism, are selected as targets of the immune system [6]. To address this question, several investigators tried to define the fine specificity of autoantibodies to intracellular antigens, by identifying the antigenic determinants (or B-cell epitopes) recognized most frequently by autoantibodies. The identification of B-cell epitopes revealed useful information on the mechanisms involved in autoantibody production and their diversification in the course of the disease, such as molecular mimicry and epitope spreading [6]. For example, in patients with SLE, at least one autoantibody specificity can be detected 1–9 years before

* Corresponding author. Tel.: +30 210 7462670; fax: +30 210 7462664.
E-mail address: agtzi@med.uoa.gr (A.G. Tzioufas).

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the clinical onset of the disease and its diagnosis. The autoimmune response is then spreads in an ordered manner to other autoantigens and the clinical onset of disease coincides with the cessation of new autoantibody specificities development [7]. The earliest autoantibodies detected in the pre-clinical period, as individuals progress toward clinical SLE were antibodies to Ro60 (mean 3.7 years before the disease onset). McClain et al. mapped the initial, pre-disease target of the anti-Ro60 autoantibody response to the region 169–180aa (TKYKQRNGWSHK) of the autoantigen [8]. This region belongs to the previously identified SLE related epitope 169–190aa by Routsias et al. [9], shares sequence homology with Ro orthologs in certain bacteria such as the region KYRQRGGWSHR from the ribonucleoprotein complex of *Mycobacterium smegmatis* and it has been reported to cross-react with a viral peptide (GGSGSGPRHRDGVRR) from the Epstein–Barr virus nuclear antigen-1 (EBNA-1) without to exhibit any sequence similarity [8].

In addition, the characterization of the epitopes of an autoantigen with high sensitivity and specificity, allows the development of immunoassays based on synthetic peptides which can be utilized as substrates for autoantibody detection. As example, fillagrin in RA is recognized by about 70 percent of the patients. Anti-fillagrin antibodies are targeting mainly post-translationally modified epitopes, containing citrulline [10]. Antibodies to cyclic citrullinated peptides (anti-CCP) are detected in RA patients' sera long before the onset of the disease and are associated with erosive disease [11]. In diagnostic grounds, when anti-CCP and RF antibodies are combined, the specificity for RA is exceeded the 95% [12].

3. Structural definition of B-cell epitopes

The B-cell epitopes are diverse in structure and immunoreactivity and thereof they are classified accordingly. On the basis of the nature of the epitope within the parental protein, they are classified as: (i) linear or continuous, consisting of sequential amino acids in the primary structure of the protein, and (ii) conformational or discontinuous epitope, formed by distant regions in the protein sequence coming together in its tertiary structure. In the majority of the epitopes characterized previously as linear, not every amino acid in the sequence is essential for antibody binding. Often, there are sequence-positions that can be successfully substituted with all the 20 naturally occurred amino acids, without any loss of immunoreactivity. In this regard, linear epitopes larger than 5–6 amino acids in length, possess also features of conformational epitopes. Moreover, as the autoantigens are organized in large ribonucleoprotein complexes the term "conformational epitope" can be referred either to epitopes comprised by amino acids distributed on its secondary, tertiary or quaternary structure. Therefore, more properly the epitopes can be divided in:

- *Primary-structure epitope* (or linear epitope), consisting of sequential amino acids. Such epitopes have been identified by synthetic peptide mapping the majority of autoantigens including Ro60, Ro52, La, SmB, SmD, RNP-70 and Scl-70 etc.
- *Secondary-structure epitope*, formed by amino acids distributed in simple three-dimensional structures, such as α-helices or β-sheets. These epitopes have been identified in PM/Scl-100 autoantigen by a combination of peptide scans and mutational analyses. In these studies, epitopes were defined as a local α-helical secondary structure stretch with all amino acids relevant for antibody binding is located at one side of the helix.
- *Tertiary-structure epitopes*, are formed by distant regions of the protein sequence, which are coming together in the tertiary structure. It has been suggested that such conformational epitopes are the main target of some autoantibodies (e.g. anti-Ro60kD).
- *Quaternary-structure epitopes*, which are consisted of amino acids distributed over different subunits within a macromolecular complex, forming a structure recognized by the autoantibody. Such epitopes have been identified in Ro/La RNP complex as well as in nucleosome subunits, composed of histones and DNA elements.
- *Cryptic epitopes (cryptotopes)*. These are usually linear epitopes hidden within the native structure of the autoantigen. They become accessible to antibody binding after disruption of the three-dimensional structure (e.g. by denaturation, proteolytic degradation or chemical modification of the autoantigen). These epitopes are observed in a number of nuclear autoantigens, such as the Ro/La RNP, where the initial Ro60 epitope (for SLE) is cryptic, masked by the binding of hY RNA.
- *Modified epitopes*. Amino acids can be post-translationally modified. Examples of these modifications include: (i) Serine, Threonine, Tyrosine phosphorylation by protein kinases, (ii) Lysine acetylation or ubiquitination, (iii) Cysteine lipidation or oxidation (disulphide-bond formation), (iv) Glutamic acid methylation or γ-carboxylation, (v) Glutamine deamidation (vi) Asparagine (N-linked) and Serine/Threonine (O-linked) glycosylation (vii) Arginine citrullination or dimethylation and (viii) Proteolytic cleavage or degradation. In some instances, side chain modifications of specific amino acids, such as citrullination of arginine residues, are responsible for epitope high-affinity binding. Such modified amino acids have been reported in a variety of human nuclear proteins, including the Sm antigens D1 and D3, and nucleolin. The identification of these modified (usually linear) epitopes requires assays that provide the amino acid in its modified form. These assays are based mainly on synthetic peptides.
- *Neoepitopes*. Neoepitopes can be the post-translationally modified epitopes but also epitopes pre-translationally modified, derived by frameshift mutations or alternative splicing of mRNA. For example, Bachmann et al. [13], identified a mutated La cDNA in patients with SS that contains a frameshift mutation in a hot spot region. This mutation resulted in premature stop codon, which is located eleven amino acids downstream of the frameshift mutation. Consequently, only the sequence of the 12 amino acid La peptide (193–204aa: MKKENKIKWKLN, neoepitope) encoded by the patient's La cDNA markedly differed from the corresponding La protein sequence.
- *Apotopes*. Apotopes are epitopes that are expressed specifically on the surface of apoptotic cells. This term is not widely accepted, since it refers to epitopes that obviously belong to one of the above described categories. However, this term has been used to describe epitopes on Ro60 that differentiate Sjögren's syndrome from SLE.
- *Mimotopes*. Mimotopes are structures that mimic unknown epitopes. They are usually defined using peptide libraries. Such mimotopes can either show close homology to an antigenic sequence of a protein (linear epitope) or, alternatively, are structural homologues with a wide variety of different type epitopes (all the conformational epitope types described previously) including epitopes belonging to non-protein molecules such as polysaccharides, lipids or nucleic acids.

4. Clinical significance of antibodies to Ro/SSA and LaSSB

Anti-Ro and anti-La antibodies are found in approximately 60–90% and 30–60% of patients with primary Sjögren's syndrome, as well as in 30–40% and 10–15% of patients with SLE, respectively [14], depending on the method used for their detection. A variety of methods have been applied for their detection. Among them RNA