CMLS, Cell. Mol. Life Sci. 59 (2002) 1280–1284
1420-682X/02/081280-05

CMLS Cellular and Molecular Life Sciences

Visions & Reflections

Peptides as DNA mimics: cross-reactivity and mimicry in systemic autoimmune diseases

S. Fournel and S. Muller *

Institut de Biologie Moléculaire et Cellulaire, UPR 9021, CNRS and Louis Pasteur University, 15 rue René Descartes, 67000 Strasbourg (France), Fax: +333 88 61 06 80, e-mail: S.Muller@ibmc.u-strasbg.fr

Received 21 February 2002; received after revision 16 May 2002; accepted 14 June 2002

Key words. Mimotopes; molecular mimicry; DNA antibodies; multi-presentation; systemic lupus erythematosus; autoimmunity.

Systemic lupus erythematosus (SLE) is a rheumatic autoimmune disease that afflicts one million Americans today. The serum from patients with SLE contains antibodies (Abs) that react with a variety of nuclear antigens. In general, their level fluctuates during the course of the disease. Anti-double-stranded (ds)DNA Abs represent a marker of the disease and have been extensively studied for several decades [1]. Several lines of evidence have led to the claim that the anti-DNA Ab response meets all the features of a T-cell-dependent, antigen-driven immune response [2]. Anti-dsDNA Abs are somatically mutated IgGs, some of which possess a high affinity for DNA. However, as with several other self-antigens, e.g., histones, mammalian dsDNA alone (i.e., uncomplexed) is not immunogenic in animals [3]. Although some bacterial DNAs appear to be immunogenic in normal mouse strains, they do not elicit antibodies cross-reacting with eukaryotic DNA [4]. Moreover, T cells are unlikely to recognize fragments of dsDNA presented in the context of class II MHC molecules. These observations have prompted intense research aiming to identify possible antigenic stimuli, of either self or foreign origin, that elicit anti-DNA Ab responses, and in some instances deleterious nephritogenic Abs. The nucleosome, the basic repeating subunit of chromatin, which contains a protein octamer of histones associated with the extranucleosomal histone H1 and DNA, appeared very early to be an important candidate. It has now been identified as a major autoantigen in SLE, driving a large part of the autoimmune response in providing the peptides that activate T cell help for anti-DNA-Ab-producing B cells [5–7].

Other studies have demonstrated non-nucleosome-related peptides that cross-react with DNA Abs. In general, these peptides have been selected using random peptide libraries (phage or chemical libraries) to screen monoclonal autoAbs. Cross-reactions between dsDNA and known self-proteins have also been investigated. Similarly, some studies have been engaged to identify determinants recognized by anti-RNA autoAbs [8]. Such strategies have been used extensively to identify mimotopes of pathological antigens for vaccine purposes [9–13]. Several review articles have been devoted to molecular mimicry in autoimmunity and its implication in the breaking of tolerance to self-antigens [14–19]. This short report will concentrate only on peptide structures and proteins recognized by anti-DNA Abs. This field of research is important because it may help identify sequences, for example in infectious agents or in altered self-antigens, that might be responsible for the anti-DNA response in autoimmune patients. On the other hand, DNA mimics might also have great potential for diagnostics and peptide-based specific therapy.

* Corresponding author.

Mimotopes recognized by anti-DNA antibodies

Considerable attention has been given to the difficult problem of identifying potential antigenic stimuli that could generate anti-dsDNA Abs. Several studies have described the screening of phage display libraries with high-affinity pathogenic anti-DNA Abs and the identification of 'DNA mimotopes'. Mario Geysen was the first to introduce the term mimotope, which defines 'a molecule able to bind to the antigen combining site of an antibody molecule, not necessarily identical with the epitope inducing the antibody, but an acceptable mimic of the essential features of the epitope' [20]. An essential prerequisite for a true mimotope of an autoepitope is its ability to elicit cross-reactive antibodies, and to induce the typical signs of the autoimmune disease. This response is important, because examples of discrepancy between antigenicity and cross-reactive immunogenicity of mimotopes have been reported [21]. An epitope and a mimotope are generally accepted to share similar structural features. Cross-reactivity is rendered possible because antigen receptors (MHC molecules, T cell receptors or paratopes of Abs) are sufficiently degenerate or flexible to accommodate distinct antigens showing atomic identity but no sequence or structure homology [22–24]. The phenomenon of induced fit provides an additional mechanism for the observed structural plasticity. However, this molecular mimicry might also be due to the fact that both epitope and mimotope bind to different Ab subsites within the same paratope [25–27].

Mimotopes for a particular antigen are normally identified by screening an Ab of known or unknown specificity with combinatorial peptide libraries where random oligonucleotide sequences have been inserted into a display vector such as the filamentous bacteriophage M13. Through a cycle of selection/rescue/amplification (biopanning), clones of Ab-bound phages can be identified, and finally sequenced to reveal the amino acid composition of the selected mimotopes. Three independent groups have used this approach to identify DNA mimotopes [28–33]. Sibille et al. [28] have tested three anti-DNA IgG2a monoclonal Abs (mAbs) called J20.8, F14.16 and F4.1, which were obtained from (NZB × NZW)F1 mice spontaneously expressing some of the symptoms and pathological characteristics of patients with SLE. Several M13 phages expressing 16-mer peptides were shown to interact with the mAbs F14.6 and J20.8 (table 1). Binding of most of these phages was inhibited by soluble DNA, suggesting that these phage-displayed sequences were DNA mimotopes. Interestingly, the sequences were different, and although the sequences specific for the F14.6 mAb shared negative charges and aromatic rings that may mimic a DNA backbone, the sequences selected with the J20.8 mAb did not have any negative charges. This suggests a different type of molecular recognition, for example that the binding occurs at different subsites within the paratope. The phage-displayed mimotopes identified with the J20.8 mAb were also recognized by serum Abs from SLE patients. Finally, injection of phage clones expressing three copies of the J20.8-specific mimotopes in non-autoimmune BALB/c mice was shown to induce anti-dsDNA IgG3 Abs, suggesting that these sequences are true mimics of certain DNA epitopic structures.

Table 1. Sequences of the different DNA mimotopes.

Abs	Origin	Mimotope	References
F14.6	(NZB × NZW) F1 mouse	XXXDCTXNT$\Phi\Phi$CQL/Y/DXE[a]	28
J20.8	(NZB × NZW) F1 mouse	I: ΘTGDCW/GY/GWG/QXXCXXXX[b]	28
		II: XXXDCXXXXWQCXXXX	28
		III: AKYSCQKTMCYGLV	28
3E10	MRL/*lpr* mouse	44-amino-acid fragment	35
Sera[c]	patients with SLE	RLTSSLRYNP	33
R4A	BALB/c mouse	D/EWD/EYS/G	29–32

[a] Φ = aromatic residues (F, Y or W); X = any.
[b] Θ = hydrophobic residues (V, L or I).
[c] Affinity-purified antibodies.

Another DNA mimotope has been characterized by the group of B. Diamond [29–32]. For screening they used an M13-phage-displayed decapeptide library and an IgG2b anti-dsDNA mouse mAb called R4A (table 1). This mAb generated from a normal BALB/c mouse causes glomerulonephritis in non-autoimmune mice. Following biopanning, a consensus DNA mimotope sequence, DWEYS, was identified. Binding of phages expressing the DWEYS sequence to the R4A Ab was inhibited by soluble DNA [29]. In addition, both the natural DWEYS peptide and the all-D dweys peptide (lowercase letters are for D-amino acid residues) inhibited the binding of R4A Ab to dsDNA. The dweys all-D peptide, but not the DWEYS parent peptide containing L-amino acid residues, inhibited the deposition of R4A in the kidney [29]. Moreover, immunization of non-autoimmune BALB/c mice with the DWEYSVWLSN peptide chemically synthesized on an eight-branched lysine backbone (MAP) elicited the production of anti-DNA Abs of IgG1 and IgG2a isotypes, and induced the classical signs of lupus [30]. The IgG anti-dsDNA Ab titers were similar to those measured in 6-month-old unprimed (NZB × NZW)F1 lupus-prone mice, and immunoglobulin deposition was present in renal glomeruli after 49 days. These data suggest that the decapeptide DWEYSVWLSN not only mimics DNA but also generates a pathogenic anti-DNA response as in SLE. These findings support the idea that in SLE, a peptide that

mimics DNA may be sufficient to trigger the breakdown of tolerance against DNA. To confirm this hypothesis, the same group has studied the T cell response to the eight-branched MAP-DWEYSVWLSN construct in immunized BALB/c mice [32]. They have demonstrated that the production of anti-dsDNA Abs was correlated with the activation of peptide-specific CD_4^+ T lymphocytes in an I-E^d MHC molecule-restricted context. Remarkably, as has also been observed in other systems [34], this response occurred only when a MAP-peptide construct, and not a monomeric peptide, was used as an immunogen. Recently, Diamond et al. also demonstrated that anti-DNA Abs recognized the sequence D/EWD/EYS/G present in the extracellular domain of the murine and human N-methyl-D-aspartate NR2 receptor. Anti-DNA Abs with this cross-reactivity have been shown to mediate apoptotic death of neurons in vivo and in vitro.

Similar results have recently been obtained by Sun et al. [33] who have identified a consensus decapeptide RLTSSLRYNP recognized by affinity-purified anti-DNA Abs from serum of patients with SLE (table 1). This work was performed using a 15-mer peptide library displayed on the gene VIII product of an fd phage for the initial screening. Eighty-eight percent of SLE sera reacted in ELISA with the consensus RLTSSLRYNP peptide presented in an 8-mer MAP construct, and this binding was competitively inhibited by ds and ssDNA as well as by native (but not denatured) RNA. Immunization of rabbits with the MAP-presented peptide induced the production of anti-peptide Abs that cross-reacted with ss and ds-DNA. All these findings suggest that the RLTSSLRYNP peptide corresponds to a mimotope of DNA and native RNA.

Another study has described the cross-reaction between a peptide sequence and anti-DNA Ab [35]. This peptide was identified with the pathogenic anti-DNA autoAb 3E10 (table 1) tested with a cDNA expression library from human placenta. In contrast to the studies reported above, this monomeric peptide of 44 residues was found to correspond to a sequence of HP8, a protein of the osteonectin/SPARC family of extracellular matrix proteins. Several experiments including mutagenesis have demonstrated that the binding of dsDNA and HP8 protein occurs through overlapping portions of the Ab-binding site.

The sequence of DNA mimotopes identified in these different studies appears to be very different (table 1). They are recognized by anti-DNA Abs of various origin and a first hypothesis may be that these peptides might bind to different Ab subsites within the paratope of these Abs. This finding may also reflect the extent of Ab degeneracy and the diversity of sequences that can be recognized in a peptide library. Affinity measurements as well as thermodynamic and structural studies should help to understand the mechanisms involved in the recognition of these DNA mimics.

Cross-reactivity of anti-DNA antibodies with known self-proteins

There is a growing body of evidence demonstrating that anti-DNA Abs can bind different self-antigens including nuclear and cytoplasmic antigens as well as cell surface proteins. For example, anti-DNA mAbs have been shown to cross-react with several ribonucleoproteins such as snRNP A and D proteins, which are also well-known targets of autoAbs in SLE [36, 37]. These mAbs react with unfolded, denatured proteins tested by Western immunoblotting and fail to react with the native U1snRNP particle in solution, in ELISA or in RNA immunoprecipitation experiments. The residues recognized within the U1A and SmD proteins by anti-DNA Abs have not been identified. Interestingly, these cross-reactive Abs were found to be pathogenic by accelerating nephritis in young lupus mice [38], and have the property of binding and in some cases penetrating cells in culture [39]. Anti-DNA autoAbs were also found to react with ribosomal P proteins, various nuclear enzymes, including DNAse I, and more recently with the protein translation factor EF-2. Other examples such as extracellular matrix/basement membrane components including α-actinin and laminin, as well as myosin I that can be exposed on the cell surface have also been described as antigen targets of anti-DNA Abs.

Repetitive epitopes in autoantigens

There is an interesting observation regarding the structure of self-antigens recognized by anti-nuclear autoAbs. They often contain repeating epitopes, either at the level of a single antigen (DNA or RNA are typical examples, as is histone H1, which contains several similar motifs all along the protein [40]) or within the macromolecular complexes targeted by autoAbs. For example, the spliceosomal U1snRNP particle, which is the target of Abs in SLE and mixed connective tissue disease, contains several proteins encompassing the well-studied PPPGMRPP motif present in several snRNPs (SmB/B′, U1A and U1C), and the RNP1 motif present in the U1-70K protein, U1A, hnRNPA2/B1, La and Ro proteins [41, 42]. This ‘multi-presentation’ enhances considerably the avidity of the interaction and may be important pathologically, for example for the spreading of the autoimmune response during the course of the disease [42].

That DNA mimotopes seem to be antigenic and immunogenic when presented as MAPs or when selected phages are used in either the ELISA or immunization protocols is a striking result. Mention is often made that the respective monomeric sequences do not exhibit the same properties. This result may be related to the repetition of epitopes in autoantigens. It may also be due to the fact that the conformation of peptides free in solution is quite different

from that of the same sequence in the MAP construct or expressed at the surface of phages. Furthermore, a multivalent binding of Abs is possible with MAPs and phages. The use of peptides presented as MAPs to enhance the level of reactivity of autoAbs in ELISA has been described by several authors [43, 44] and this system of multiple presentation effectively possesses many advantages. However, there are some important limitations to keep in mind (e.g., higher background in ELISA, possible false-positive reactions, lower solubility compared to monomeric peptides) [45]. Thus, in the case of autoAbs, this strategy has to be used with caution. In this regard, Harley and James have found that immunization of various types of animal with the PPPGMRPP peptide from SmB/B′ synthesized as MAP, but not with the monomeric peptide, was associated in rabbits with the development of symptoms reminiscent of SLE [46].

Concluding remarks

The literature describes many examples of molecular mimicry between proteins, based on a degree of sequence homology or structural similarity. Molecular mimicry between DNA (or RNA) and protein, as well as mimicry between sugar and proteins [47], may result from other mechanisms. Despite the fact that nucleic acids (or carbohydrates) and proteins are chemically very different and are not expected to form the same shapes or to present the same chemical moieties on their surface, they might present identical or overlapping topological surfaces, which can be recognized by the same antibodies [8, 22, 48]. Autoantibodies are often polyreactive in nature; whether this property is associated with particular pathogenic functions is still unclear. This does not mean that polyreactive Abs are only low-affinity IgMs, or are 'sticky' polyspecific Abs. Polyreactive IgGs with relatively high affinities for a few antigens can also be readily detected in the serum of autoimmune (and normal) individuals. Although the molecular mechanisms involved in this phenomenon of apparent polyreactivity have not been clearly elucidated (true molecular similarity of antigens, unmasking effect of hidden antibodies or binding to subsites within the paratope [49, 50]), this particular feature is certainly favorable for identifying mimotopes of important autoantigens.

If the peptides identified with anti-DNA Abs represent true mimotopes rather than linear epitopes of genuine autoantigen(s), this will unfortunately not help to identify causative antigens, for example of microbial origin, that might be responsible for the anti-DNA response in autoimmune individuals. However, peptide mimics of DNA may have important applications in diagnostic assays and in therapeutic peptide strategies based on immunomodulation of the autoimmune response. Such mimotopes could be used to restore tolerance and if they contain T cell epitopes, modified peptides with favorable pharmacokinetic properties might play the role of altered peptide ligands of T cell receptors [51, 52]. This strategy may be important to change the cytokine patterns secreted by T cells and consequently influence the nature of T cell help to B cells producing anti-DNA Abs [53]. Because replacing natural peptides by peptide analogues, which are more resistant to proteolytic digestion in the body fluids, is possible in certain cases [52, 54], peptide mimics of DNA certainly represent important tools to consider for future therapeutic strategies.

Acknowledgements. We thank Dr Harry Partidos (UPR 9021 CNRS, Strasbourg) and Dr Marc Monestier (Temple University School of Medicine at Philadelphia) for critical reading of the manuscript and for stimulating discussions.

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