Clinical and Experimental Rheumatology 2002; 20: 289-291.

Epitopes and complementary epitopes of autoantigens: Candidate probes to study and modulate the autoimmune response

A.G. Tzioufas
H.M. Moutsopoulos

Department of Pathophysiology, School of Medicine, University of Athens, Athens, Greece

Athanasios G. Tzioufas, MD; Haralampos M. Moutsopoulos, MD, Professor of Medicine.

Please address correspondence to:
Athanasios G. Tzioufas, MD,
Department of Pathophysiology,
School of Medicine, University of Athens,
75 M. Asias st, 11527 Athens, Greece.
E-mail: agtzi@med.uoa.gr

Received on May 10, 2002; accepted on May 13, 2002.

Key words: Autoantibodies, epitopes, complementary peptides, idiotypes.

The key element in autoimmune diseases is the production of autoreactive B- and T- lymphocytes directed against different organ or non-organ specific autoantigens. The later are, in their vast majority, large subcellular complexes composed of a number of proteins, non-covalently associated with nucleic acids. Antibodies against nucleosomes, a complex of double stranded DNA and histones, are closely associated with systemic lupus erythematosus; antibodies against the spliceosome (Sm and U1 RNP) are found in SLE and mixed connective disease, while antibodies to cytoplasmic ribonucleoproteins (also referred to as hy or Ro RNPs) are usually deteted in primary Sjögren's syndrome and SLE (1).

After the definition of the autoantibodies and their initial clinical correlations in the 1970s and early 1980s, a number of investigators tried to define the fine specificity of autoantibodies (B cell epitopes) and to a lesser extent that of autoreactive T cells (T cell epitopes) (2-4). The concept and the expectations of this endeavour were simple – that the identification of major epitope(s) would allow researchers to: a) define more homogenous disease subgroups, b) study in depth the structure and the biologic properties of a given epitope, c) develop assays with higher sensitivity and specificity for autoantibody detection, and d) create therapeutic tools targeting highly specific structures within the moiety of the autoantigens.

The B cell epitopes have been studied more extensively, since the handling of autoantibodies is easier compared to autoreactive T cells and they are generously offered in large amounts on the laboratory bench from patients'sera. B cell epitopes can be either linear, formed by adjacent aminoacid residues, in the primary structure of a protein, or conformational, consisting of amino acid residues from distant regions in the sequence of a given protein that are spatially juxtaposed upon folding. Diverse approaches have been used to map the B cell epitopes. These include proteolytic degradation of the native autoantigen, preparation of recombinant fragments of the autoantigen and testing of the corresponding autoantibodies, and finally the synthesis of multiple overlapping peptides covering the whole sequence of the autoantigen and subsequent determination of the immunoreactive peptides and the minimum required length for autoantibody binding. The first two techniques detect mainly conformational epitopes, while the method with the synthetic peptides reveals primarily linear epitopes.

Using these methods several laboratories have provided conflicting results with regard to epitope mapping of intracellular autoantigens. All of these studies have pointed out, however, that: a) there is no currently available single approach which can be used for the identification of all epitopes in a given autoantigen, b) sera from patients contain autoantibodies against multiple different linear and conformational epitopes, and c) these antibodies are of the IgG class, a finding which denotes that their production is T-cell dependent (4). On the other hand antibodies against certain epitopes can serve as diagnostic tools. A classic example is the common epitope of the ribosomal P proteins located in the C-terminal portion. Antibodies against this peptide are associated with neuropsychiatric lupus (5). Other epitopes, such as for example the sequence spanning the region 349-364aa of La/SSB (6) or the shared epitope PPGMRPP of Sm autoantigen (7), when used as substrates for autoantibody detection present high disease specificity and sensitivity, similar or even better to those observed using the conventional methods for autoantibody detection. In addition, antibodies targeting certain epitopes of La/SSB are linked with the HLA haplotypes in Sjögren's syndrome much more strongly as compared to the whole population of anti-La/SSB autoantibodies (8).

Structural studies of the epitopes of autoantigens revealed homologous sequences shared with other foreign or self proteins. These homologous primary sequences have putative 3-D structures capable of reacting with the same antibody. This phenomenon is called molecular mimicry. Several regions of different autoantigens, including Ro60KD, La/SSB, SmD, SmBB and U1RNP70KD, present sequence

similarity with structures within viral proteins. Nevertheless, not all individuals infected with the specific viruses have autoimmune reactions (4). On the other hand, careful studies in human T cell clones or experimental animals which investigate in detail paired immune responses against peptides with molecular mimicry are lacking.

Knowledge of the structure of autoepitopes is helpful for understanding the mechanism(s) through which autoimmune responses are triggered and autoantibodies are generated. Studies in sequential human sera from patients with SLE and immunization of experimental animals with the epitopes disclosed the following findings: (a) Early in the course of the disease or the immunization dates, the autoantibody response is limited and directed against particular epitopes. With time it expands, involving neighboring or even distant epitopes within the complex of the autoantigen; this phenomenon is called epitope spreading (9, 10). (b) The presence of putative T cell epitopes on the autoantigens (11,12) which are mainly responsible for the initiation, augmentation and perpetuation of the abberant autoimmune reactivity. Thus, it appears that the autoimmune response is a dynamic, antigen-driven process very similar to that observed in the specific immune response against foreign antigens.

The regulation of the autoimmune response is achieved through different mechanisms including: a) an extrinsic mechanism involving an antigen, and b) internal regulatory mechanisms such as the antiidiotypic response. In this puzzle, knowledge of the primary sequence of given autoepitopes capable of inducing a "full blown" autoimmune response is of vital importance. Ideally, the epitopes themselves or their antagonists can be used not only to study in detail the autoimmune response, but also to design and create rational antigen specific therapeutic interventions.

One of the major challenges for protein biochemistry is to design molecules to interact specifically with given sites of interest on biologically important proteins. The evolving recognition of the role of patterning of hydropathy in protein folding and shape suggested a way to design proteins or peptides with complementary contours by merely inverting the hydropathic codes (13). In this respect, if the architecture of a peptide or protein is resolved by its pattern of hydropathy, then exactly inverting a particular pattern or code may resultin a second peptide or protein with a complementary surface contourto the first since the hydrophobic effect is involved but in a reversedorientation. Inversion of the hydropathic pattern of one sequence relative to another can be achieved by computer programs designed for this task or by simple relianceon an interesting characteristic of the genetic code. In the later instance, since A and U are complementary and in the second codon position specify hydrophilic and hydrophobic R groups, respectively, and considering that second base G and C generally encode slightly hydrophilic R groups, amino acid sequences deciphered from noncoding strands of DNA will have exactly inverted patternsof hydropathy relative to those of the coding strands. Such peptides specified by complementary nucleotide sequences or designed by simply inverting the hydropathic pattern are termed complementary peptides and have characteristics suggestive of complementary structure (14).

More than 40 different systems of complementary peptides that bind one another with specificity and various affinities have been described. Additional evidence of complementary structure include the ability to: locate the interactive sites of ligands and receptors by the identification of complementary sequences; generate interacting pairs of monoclonal idiotypic and anti-idiotypic antibodies with complementary combining sites by immunization with pairs of complementary peptides; and produce antibodies to receptor-binding sites by immunizationwith complementary peptides for the receptor's ligand (14, 15).

In the last year an effort has been undertaken in our laboratory to investigate the relevance and biologic significance of complementary peptides corresponding to major epitopes of La/SSB. Two synthetic peptides corresponding to epitopes A^{289}NNGNLQLRNKEVTWEVLEG^{308} (pep289-308) and G^{349}SGKGKVQFQG-KKTKF^{364} (pep349-364) of La/SSB autoantigen, and their complementary peptides deduced from the antisense RNA corresponding to these epitopes (cpep 289-308 and cpep 349-364, respectively) have been used. The main findings of this study (16) include: a) both epitopes and complementary epitopes reacted with sera containing anti-La/SSB antibodies,b) antibodies to complementary epitopes were antiidiotypic antibodies to anti-epitope (anti-La/SSB) antibodies, and c) in autoimmune sera negative by the conventional methods for anti-La/SSB antibodies, the antiidiotypic antibodies bound and masked the idiotype (anti-La/SSB) antibodies. Using the complementary epitopes as inhibitors of the anti-idiotypic antibodies, the anti-La/SSB reactivity was recovered.

The presence of anti-complementary epitope antibodies and their anti-idiotypic association with the autoantibodies generate a "chicken and egg" principle. Are the anti-complementary epitope antibodies an antiidiotypic response to autoantibodies? Or they are the initiating agent for the formation of autoantibodies ? In this respect, anti-complementary epitope antibodies could be generated as a response against a protein bearing the complementary epitope sequence. This protein can be produced by reverse strand transcription and translation, which has been recently demonstrated as an alternative mechanism for gene expression in viral infected cells. Alternatively, antibodies to complementary epitopes may occur as a result of molecular mimicry between a host protein and an infectious agent sharing structures resembling the complementary epitope. Finally, these antibodies may arise as a response to a protein with autoantigen binding properties.

Whatever the answer is to these intriguing questions, these observations point out the chaotic course of the autoimmune response in an established disease. On the other hand, the handling of the elements which participate in the autoimmune response becomes easier when using as probes epitopes and

their complementary epitopes. Thus, the manipulation of this network, targeting major epitopes of the autoantigens which apparently are involved in the initiation of the autoimmune response in selected experimental animals or even in the human material of patients at the earliest stages of the disease, may provide useful insights into the mechanisms of systemic autoimmunity and indicate directions for therapeutic intervention.

References

1. NAPARSTEK Y, PLOTZ PH: The role of autoantibodies in autoimmune disease. *Annu Rev Immunol* 1993; 11: 79-104.
2. SCOFIELD RH, FARRIS AD, HORSFALL AC, HARLEY JB: Fine specificity of the autoimmune response to the Ro/SSA and La/SSB ribonucleoproteins. *Arthritis Rheum* 1999; 42: 199-209.
3. WAHREN-HERLENIUS M, MULLER S, ISENBERG D: Analysis of B-cell epitopes of the Ro/SS-A autoantigen. *Immunol Today* 1999; 20: 234-40.
4. MOUTSOPOULOS NM, ROUTSIAS JG, VLACHOYIANNOPOULOS PG, TZIOUFAS AG, MOUTSOPOULOS HM: B-cell epitopes of intracellular autoantigens: Myth and reality. *Mol Medicine* 2000; 6: 141-51.
5. TZIOUFAS AG, TZORTZAKIS NG, PANOUPOMONIS E *et al.*: The clinical relevance of antibodies to ribosomal-P common epitope in two targeted systemic lupus erythematosus populations: A large cohort of consecutive patients and patients with active central nervous system disease. *Ann Rheum Dis* 2000; 99-104.
6. YIANNAKI EE, TZIOUFAS AG, BACHMANN M *et al.*: The value of synthetic linear epitope analogues of La/SSB for the detection of autoantibodies to La/SSB; specificity, sensitivity and comparison of methods. *Clin Exp Immunol* 1998; 112: 152-8.
7. PETROVAS CJ, VLACHOYIANNOPOULOS PG, TZIOUFAS AG *et al.*: A major Sm epitope enchored to sequential oligopeptide carriers is a suitable antigenic substrate to detect anti-Sm antibodies. *J Immunol Methods* 1998; 220: 59-68.
8. TZIOUFAS AG, WASSMUTH R, DAFNI UG *et al.*: Clinical, immunologic and immunogenetic aspects of autoantibody production against Ro/SSA, La/SSB and their linear epitopes in primary Sjögren's syndrome (pSS): A European multicenter study. *Ann Rheum Dis* 2002; 61: 398-404.
9. MCCLUSKEY J, FARRIS AD, KEECH CL *et al.*: Determinant spreading:lessons from animal models and human disease. *Immunol Rev* 1998; 164: 209-29.
10. FATENEJAD S, MAMULA MJ, CRAFT J: Role of intermolecular/intrastructural B- and T-cell determinants in the diversification of autoantibodies to ribonucleoprotein particles. *Proc Natl Acad Sci USA* 1993; 90: 12010-14.
11. REYNOLDS P, GORDON TP, PURCELL AW, JACKSON DC, MCCLUSKEY J: Hierarchical self-tolerance to T cell determinants within the ubiquitous nuclear self-antigen La (SS-B) permits induction of systemic autoimmunity in normal mice. *J Exp Med* 1996; 184:1857-70.
12. YIANNAKI E, VLACHOYIANNOPOULOS PG, MANOUSSAKIS MN *et al.*: Study of antibody and T-cell responses in rabbits immunized with synthetic human B cell epitope analogues of La (SSB) autoantigen. *Clin Exp Immunol* 2000; 121: 1-7.
13. BOST KL, BLALOCK JE: Preparation and use of complementary peptides. *Methods Enzymol* 1989; 168: 16-28.
14. BLALOCK JE: Genetic origins of protein shape and interaction rules. *Nat Med* 1995 1: 876-8.
15. VILLAIN M, JACKSON PL, MANION MK *et al.*: *De novo* design of peptides targeted to the EF hands of calmodulin. *J Biol Chem* 2000; 275: 2676-85.
16. ROUTSIAS JG, TOULOUPI E, DOTSIKA E *et al.*: Unmasking the anti-La/SSB response in sera from patients with Sjögren's syndrome by specific blocking of anti-idiotypic antibodies to La/SSB antigenic determinants. *Mol Med* 2002 (in press).
17. VAN DEN EYDDE BJGB, PROBST-KEPPER M, MICHAUX L *et al.*: A new antigen recognized by cytosolic T lymphocytes on a human kidney tumor results from reverse strand transcription. *J Exp Med* 2000; 190: 1793-9.