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

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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 detected 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 specificity 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 aminacid 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 autoanti-bodies, 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 individu-
als 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 autoepi-
topes is helpful for understanding the
mechanism(s) through which autoim-
mune responses are triggered and auto-
antibodies are generated. Studies in
sequential human sera from patients
with SLE and immunization of experi-
mental animals with the epitopes dis-
closed 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 ex-
pands, 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 re-
sponse 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 differ-
tent 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 se-
quence of given autoepitopes capable
of inducing a “full blown” autoimmune
response is of vital importance. Ideally,
the epitopes themselves or their antago-
nists can be used not only to study in
detail the autoimmune response, but
also to design and create rational anti-
gen 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 pro-
teins. The evolving recognition of the
role of patterning of hydrophathy in pro-
tein folding and shape suggested a way
to design proteins or peptides with com-
plementary contours by merely inver-
ting the hydrophatic codes (13). In this
respect, if the architecture of a peptide
or protein is resolved by its pattern
of hydrophathy, then exactly inverting a
particular pattern or code may result
in a second peptide or protein with a com-
plementary surface contour to the first
since the hydrophobic effect is involved
but in a reversedorientation. Inver-
sion of the hydrophatic pattern of one
sequence relative to another can be
achieved by computer programs de-
signed for this task or by simple re-
tlanceon 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 sec-
ond base G and C generally encode
slightly hydrophilic R groups, amino
acid sequences deciphered from non-
coding strands of DNA will have exactly
inverted patternsof hydrophathy rela-
tive to those of the coding strands. Such
peptides specified by complementary
nucleotide sequences or designed by
simply inverting the hydropathic pat-
tern are termed complementary pep-
tides and have characteristics sugge-
tive of complementary structure (14).
More than 40 different systems of com-
plementary peptides that bind one
another with specificity and various
affinities have been described. Ad-
ditional evidence of complementary struc-
ture include the ability to: locate the
interactive sites of ligands and recep-
tors by the identification of comple-
mentary sequences; generate interact-
ing pairs of monoclonal idiotypic and
anti-idiotypic antibodies with comple-
mentary combining sites by immuniza-
tion with pairs of complementary pep-
tides; and produce antibodies to recep-
tor-binding sites by immunization with
complementary peptides for the recep-
tor’s ligand (14, 15).
In the last year an effort has been
undertaken in our laboratory to investi-
gate the relevance and biologic signifi-
cance of complementary peptides corre-
sponding to epitopes A280-308
PGNGLQL-RNKVTVWLEG308 and
G104-SGKGVQG-364
SSB. Two synthetic peptides corre-
sponding to epitopes A280-308
PGNGLQL-RNKVTVWLEG308 and
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SSB. Two synthetic peptides corre-


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