Use of synthetic peptides for the detection and quantification of autoantibodies

Keith B. Elkon
The Hospital for Special Surgery-Cornell University Medical College, New York, NY 10021, USA

Key words: autoantibody, autoantigen, synthetic peptide

Abbreviations: r: recombinant, SLE: systemic lupus erythematosus

The characteristics of the humoral immune response to protein autoantigens have been reviewed recently [1,2]. Most of the autoantibodies demonstrate properties similar to those described for a secondary immune response against foreign protein antigens. Briefly, the autoantibodies are polyclonal, directed against multiple epitopes, react predominantly with conformational epitopes, are composed of the IgG isotype, IgG1 and 3 subclasses (although exceptions are noted [3]), and frequently bind to multiple components within the same RNP particle. The signal difference between autoantibodies and antibodies to foreign proteins is the selection of epitopes — autoantibodies usually bind to highly conserved epitopes on self proteins [1,2] whereas antibodies to foreign proteins select epitopes most different from self [4].

X-ray diffraction studies of monoclonal antibody-antigen complexes indicate that the antibodies contact 15–22 amino acids on several loops on the surface of the antigen [reviewed in 5]. Despite this fact, some antisera to foreign or self antigens contain populations of antibodies that also recognize short peptides (‘anti-peptide antibodies’). Peptide antigens are therefore thought to correspond either to part of a conformational epitope or to the less ordered structure of the termini of the protein. The distinction between a conformational (discontinuous/assembled) or linear (continuous) epitope is made operationally by epitope mapping (see this volume). Antibodies that bind to a short peptide define a linear epitope whereas, when antibodies fail to bind to fragments of the protein antigen, these antibodies are presumed to be directed against amino acids that are located on different parts of the protein but are apposed during folding of the molecule (conformational/discontinuous epitope). This distinction is of course not entirely accurate since even small peptides may assume a more complex tertiary structure under appropriate experimental conditions [6]. The peptides may also adopt different conformations in solution, of which only a small number are recognized by the antibody [7].

Once a linear epitope has been defined, a synthetic peptide antigen can be synthesized. Since, for the reasons mentioned above, the antibody is likely to bind to the synthetic peptide with low affinity [8], it is important to test the antibody for binding to a recombinant peptide or peptide obtained by partial proteolysis under denaturing conditions (eg. by Western blotting or by ELISA). If the antibody retains binding to the denatured peptide, it is reasonable to consider synthesizing a peptide. When epitope mapping has been performed with overlapping short synthetic peptides, only the boundaries and length of the peptide require consideration. Greater specificity is obtained with longer (>20 amino acids) synthetic peptides.
Advantages and potential disadvantages of synthetic peptides as antigens

Solid-phase immunoassays (RIA, ELISA) utilizing synthetic peptides as antigens are amongst the most sensitive and specific assays currently available for autoantibody detection. Most importantly, these assays are quantitative. In addition, the peptides are synthesized in milligram amounts. Since these reagents are essentially pure, they provide an ideal source of antigen for diagnostic and experimental use. Although recombinant (r) proteins also offer unlimited supplies of antigen, “purified” preparations are frequently contaminated with E. coli proteins which may produce false positive results in immunoassays. Furthermore, most r autoantigens have been synthesized as hybrid proteins fused to an E. coli protein (eg. β-galactosidase, TrpE).

Disadvantages of synthetic peptide antigens are the initial expense of peptide synthesis and possibilities for false positive results. False positive results may occur because the peptide shares amino acid sequence homology with other foreign or self antigens (cross-reactivity can occur with homologous tripeptide sequences [9]) or because chemical conjugation alters the antigenicity of the peptide. A third potential disadvantage of synthetic peptide antigens is the absence of posttranslational modifications, although an absolute requirement for autoantibody binding to posttranslationally modified protein antigen has not yet been reported.

Peptide synthesis

Methods for chemical synthesis of peptides are beyond the scope of this review and are discussed elsewhere [10]. Some practical considerations should, however, be kept in mind. Chemical synthesis of peptides is an expensive and slow process. For this reason, it is advantageous to map the location of the epitope as precisely as possible prior to synthesis. When the smallest fragment containing the epitope has been identified, it may be possible to further narrow down the location of the epitope by using computer algorithms. Programs that utilize the primary amino acid sequence to calculate the hydrophobicity, flexibility or predicted secondary structure of the protein have been claimed to accurately predict the location of epitopes. However, as discussed elsewhere [11,12], these programs have, at best, a 60% success rate. These limitations are largely due to the relatively poor predictive power of the algorithms for secondary structure derived from the primary sequence of the protein [13]. Insufficient information is available on the accuracy of computer prediction of epitopes on autoantigens. However, since autoantibodies may have additional constraints induced by tolerance, it is unlikely that these algorithms will have a higher success rate with autoantigens. Correlations between the predicted and actual epitopes have been accurate for the systemic lupus erythematosus antigen, P2 but not for La (SSB) or Sm (see below).

Once synthesized the peptides should, at minimum, be tested for homogeneity by HPLC and for amino acid composition. The peptides can either be applied directly or, following conjugation to non-antigenic proteins (BSA, OVA), to plastic microtiter plates. We favor the latter approach since binding to the plate does not depend on the solubility of the peptide and less peptide is used per assay. It is very important to verify that the peptide adsorbs to the microtiter plate (direct assay) or is conjugated to the carrier protein in the expected molar ratio.

The remainder of this review will focus on the utilization of synthetic peptides for the detection and quantification of autoantibodies in human autoimmune diseases. Application of synthetic peptide antigens in murine lupus [14–16] and the use of synthetic peptides for epitope mapping (this volume) are discussed elsewhere.

Organ specific autoimmune diseases

Myasthenia gravis

Anti-acetylcholine receptor (AChR) antibodies in myasthenia gravis preferentially bind to conformational epitopes on the z subunit of the AChR