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Peptide epitope mapping in vaccine development: Introduction

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Protection from infectious disease by the host immune response requires specific molecular recognition of unique antigenic determinants of a given pathogen. An epitope is an antigenic determinant which: 1) specifically stimulates the immune response (either B or T cell mediated); and 2) is acted upon by the products of these protective mechanisms. In B cell immunity, antibodies produced from stimulation by specific epitopes recognize and bind to these same antigenic structures. Identification of protective epitopes is extremely valuable to successful vaccine development. In order to be protective these antibodies must, in addition to recognition and binding, interfere with some vital step in pathogenesis such as adherence or toxin action. Protein B cell epitopes are frequently composed of the side chains (R-groups) of the amino acids found at solvent-exposed surfaces. These epitopes are classified as *continuous* (also linear or sequential) if composed of a single antibody-recognizing element located at a single locus of the primary structure. They are *discontinuous* (or assembled) if more than one physically separated entity is involved. T cell epitopes are peptides on the surface of antigen-presenting cells (macrophages, dendritic cells, and B cells) that are bound to major histocompatibility proteins; the T cell recognizes this peptide-MHC complex.

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Identification and characterization of epitopes is clearly important in vaccine design strategy. This was extremely difficult prior to the development of the tethered peptide pin assay of Geysen and coworkers [8], a technique particularly well suited to the identification and structural characterization of protein B cell epitopes, and T cell epitopes [10]. In these procedures, peptides representing primary structure segments of the protein antigen of interest are synthesized immobilized on polypropylene pins. Specific details of the Geysen pin method are described elsewhere [2,5,8,11], however the B cell epitope procedure can be summarized as follows. For details on the T cell epitope analysis procedure refer to [10,11].

(a) Chemical synthesis of peptides immobilized on polypropylene pins

These peptides are frequently 8- to 12-mers for B cell analysis. There is significant sequence overlap from pin to pin, with an offset of a single amino acid (or more if the total number of peptides synthesized is too large). Pins are organized to fit a 96-well plate format. Either a selected portion of a protein or the entire length of the protein primary sequence is represented by the synthesized peptides.

(b) Antibody capture ELISA

Sera (polyclonal or monoclonal) known to react with the protein antigen in question are used in an antibody capture ELISA in which the tethered peptides serve as antigen. A labeled secondary antibody is employed to give a color reaction which is then read in a plate reader.

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(c) Interpretation

Analysis of results gives a map with general epitope-containing regions identified. The intensity of reaction, as well as frequency of reaction in sera from outbred animals, indicates immunodominant regions.

(d) Building on initial results

After the general synthesis described above, additional sets of peptides can be synthesized in order to identify the size of the epitope (window net), as well as identify the contribution of each amino acid in the epitope to antibody binding (replacement net). The peptide length is varied in the window net strategy, with all of the amino acids from epitope regions included in successive syntheses of 4-, 5-, 6-, 7-, and 8-amino acid peptides. In the replacement net strategy, residues essential to the epitope may be determined by synthesizing peptides in which a single amino acid of the epitope is replaced by other amino acids. A series of peptides is generated and tested with positive antibody for each amino acid of the original epitope.

Information obtained from the Geysen pin test may be applied in a number of ways in vaccine design, including determination of protective epitopes. This is done by first conjugating epitope-specific peptides to carrier proteins. The sera (again polyclonal or monoclonal) produced by animals immunized with this material frequently recognize native protein antigen as well as the immunizing peptide. These sera can be tested for the ability to neutralize a specific virulence mechanism (adhesion of pili, for example) *in vitro*, followed by animal protection studies, to determine potentially-protective epitopes. Overall, this information provides the ability to produce a vaccine which targets the immune response to relevant regions of the antigen while ignoring regions which may be immunogenic but unimportant in pathogenesis.

Papers presented in this symposium clearly demonstrate

the power of this methdology. Two significant modifications of the Geysen pin test are described by Loomis-Price and others [9], which reduce greatly the background of human antibodies produced in response to HIV infection and in response to immunization with gp 160. Studies by Cassels *et al* [3], show the linear B and T cell epitopes of CFA/I, a pilus from enterotoxigenic *Escherichia coli*, as well as demonstrate that a peptide derived from the epitope-rich N-terminus of CFA/I can induce a cross-reactive antibody response to related pilus proteins. Zhong *et al* [13], compare the pin method with an alternative epitope analysis technique, random phage peptide display libraries, in the utilization of neutralizing monoclonal antibodies for analysis of epitopes of the pathogen, *Chlamydia trachomatis*. Information relating to presentations by the other speakers of the session can be found in the program abstracts, as well as in references for Drs Geysen [7,8,10], Castric [4], and Tainer [6,12].

Peptide vaccines are currently under development to many infectious diseases. Peptide vaccine approaches to cholera, shigella, malaria, influenza, and HIV appear to be particularly promising [1]. It is hoped that from an examination of one particularly powerful methodology to identify B and T cell epitopes, and its application in infectious disease, that future research will benefit and lead to safe and efficacious vaccines.

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