

Applications Broaden for Epitope Mapping

Basic Tool Has Copious Uses in Development of Vaccines and Protein Therapeutics

K. John Morrow Jr., Ph.D.

Epitope mapping, initially a basic-science tool for understanding the 3-D interactions between antigens and antibodies, is now being extensively employed to develop new therapeutics. In vaccine technology, especially, scientists in academia and biotech firms have mobilized epitope discovery based on the use of high-throughput antibody screening for rational vaccine design.

There are two arms to the immune system—the humoral or B-cell response and the cell-based or T-cell response. B-cell bioinformatics studies have lagged behind, thus many of the most important discoveries have targeted the T-cell system. This is because T-cell epitopes are bound in a linear form to the HLA receptor, allowing the interface between ligand and T-cell to be modeled with unparalleled accuracy.

A vast, uncharted landscape of other applications abounds. According to Robert Ladner, Ph.D., svp and CTO of Dyax (www.dyax.com), epitope mapping can be a starting point in the discovery of the structure of an antigen and design of an appropriate mimic, understanding autoimmune diseases, and connecting a SNP polymorphism to a specific protein structure.

Computational methods for B-cell epitope mapping are based on the ability of the specific antibody to affinity-isolate specific short peptides from combinatorial phage-display libraries. These peptides serve as leads for the elucidation of the epitope corresponding to the screening antibody. The recognition of these epitopes on viral or bacterial targets could permit the development of vaccines that would consist of the epitope only, a strategy that could be much safer yet just as effective.

Furthermore, epitope characterization is vital for rational drug design; in many cases a solved 3-D structure for an antigen-antibody interaction is unavailable.

A number of computational algorithms are now obtainable for mapping conformational discontinuous epitopes. This partnership of in silico and in vitro approaches constitutes a powerful toolbox for generating new vaccines.

Use of Algorithms

Rational vaccine and drug design demands an understanding of the molecular recognition of the antibody for the epitope region on the therapeutic target. Jonathan Gershoni, Ph.D., professor in the department of cell research and immunology, and his colleagues at Tel Aviv University are actively developing computer tools for drug design and vaccine investigations.

Ordinarily, epitope mapping can be carried out using classical phage display, screening for peptides that bind to the antibody of interest. These affinity-selected

peptides are presumed to model the 3-D structure of the authentic epitope. This resemblance, however, breaks down for those epitopes that are discontinuous, in which distantly separated amino acid sequences are brought together by the folding of a linear chain. In these situations, advanced computational methods are required to correctly infer the epitope from the designated peptides.

Dr. Gershoni and his colleagues designed

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tical analysis of the ability of Pepitope to predict authentic residues as compared to random guessing is performed, the results are highly significant.

“While some residues are missed in each prediction,” Dr. Gershoni says, “the results allow us to focus our attention to the correct surface relevant to the antibody and

cellular cytotoxicity.

The Genmab team postulated that the complement-dependent cytotoxicity potency observed was the result of the slow dissociation of these antibodies from the CD20 molecules. This feature triggers the destruction of tumor cells and makes Rituximab and HuMax CD20 effective in the treatment of B cell malignancies.

The Genmab researchers studied the extracellular loops of amino acids in CD20 that had been highly mutagenized in order to produce a number of amino acid variants. This allowed them to determine the critical binding sites on the molecule.

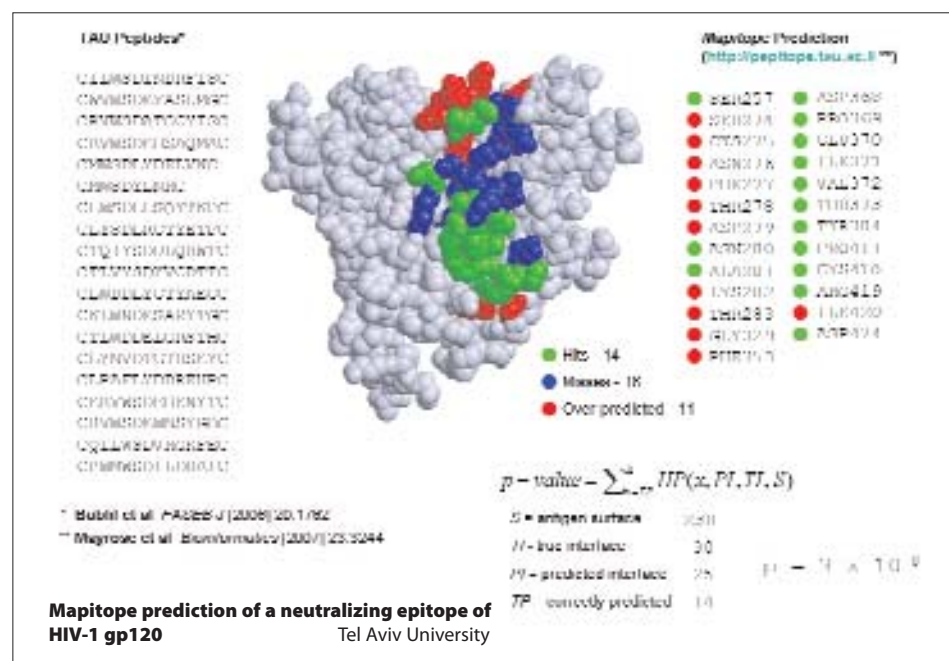
In addition, they synthesized a collection of overlapping peptides for the peptide mapping of the CD20 molecule. These peptides were immobilized and the binding of various antibodies was assessed using the Pepsan (www.pepsanpresto.com) platform.

Studies revealed that while offrate is an important factor in determining antibody potency, it is not the critical factor. Antibodies can recognize a linear string of amino acids, but they frequently bind to discontinuous epitopes. In such cases, binding is lost when membrane proteins are disrupted with detergents. So it appears that the potency of the human mAbs and their significance for cancer treatment results from the fine specificity of the molecular interactions, rather than simple binding parameters.

The relevance for product development of these sophisticated yet basic-science



Characterization of human antibody therapeutics in novel cellular models is performed by Genmab scientists.



the web-based Pepitope server (pepitope.tau.ac.il), which combines three algorithms for epitope mapping: Pepsurf, Mapiotope, and a combination of the two. Pepitope allows the alignment of short, hypothetical sequences, enabling the prediction of discontinuous epitopes based on affinity-selected peptides.

The Tel Aviv group recently compared their results with a known epitope on the well-known protein Factor VIII. The Pepitope output predicted an epitope composed of 30 residues, of which 12 are part of the genuine epitope, constituting a strong vindication of the algorithm. When a statis-

use this information for the next step in vaccine design.”

Refining Therapeutic Antibodies

Epitope mapping has been adopted by Genmab (www.genmab.com) to understand the interactions between a number of anticancer antibodies and CD20, a component of an ion channel expressed on normal and malignant B cells. This protein has emerged as one of the most significant targets for antibody immunotherapy due to the fact that antibody binding allows efficient effector recruitment and initiation of



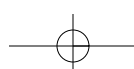
Epivax uses its technology to aid clients in the design of vaccines and nonimmunogenic therapeutic proteins.

investigations is demonstrated by the fact that one of the antibodies that binds to the small loop of the CD20 molecule, located close to the cell surface, gave superior pre-clinical activity and has now moved into advanced clinical trials for follicular lymphoma, chronic lymphocytic leukemia, and rheumatoid arthritis, according to Jan G.J. van de Winkel, Ph.D., evp and CSO.

In addition to cancer and inflammatory diseases, Genmab has two infectious disease studies in progress. “We have new antibody programs targeting HIV and hepatitis C, and we will attempt to move the hepC antibody into clinical evaluation this year,” he adds. “There is great interest in therapies that could prevent reinfection of transplant-livers in hepC patients, so we are particularly enthusiastic about this approach.”

In Silico Technologies

Scientists from Brown University’s TB/HIV research lab founded Epivax



(www.epivax.com) in 1998 with the purpose of designing computational immunological tools for vaccine development. This includes the EpiMatrix system of predictive algorithms and coefficient sets, which over the years, have been substantially revised and extended.

Epivax currently offers its technology to clients to aid in the design of effective vaccines and nonimmunogenic therapeutic proteins. The firm has used its program for T-cell epitope identification to construct an improved tularemia vaccine that is being evaluated by NIH.

The EpiMatrix System is an Oracle database used to store protein sequences and analytical outputs intended for a high-throughput environment in which preliminary analyses are processed for critical reporting applications. Protein sequences can be 9 mer peptides for example, whose affinity can be predicted against a panel of MHC class I and/or class II alleles. The EpiMatrix System's ability to rate putative epitopes on a common scale simplifies the process of selecting putative epitopes for in-vitro testing.

Epivax also has designed software for BLASTing protein sequences. BLAST is an algorithm that compares the amino acid sequences of different proteins. The Epivax technology analyzes cluster sequences or the individual 9 mer peptides against various protein databases.

Stored BLAST results can be displayed as alignments or in a summarized form. For example, human-like peptides may make poor vaccine components, since the appropriate matching T cells are likely to have been either deleted or anergized, unable to mount a complete response against their target. In the deimmunization context, identifying natural variation may help to identify substitutions that are well tolerated.

Epivax' Vaccine Computer-Assisted Design (VaccineCAD) minimizes the emergence of pseudoepitopes at the junction where two peptide sequences join together. VaccineCAD iteratively reorders epitopes so as to maximally reduce junctional immunogenicity and also introduces spacers where necessary, according to CEO and CSO, Annie de Groot, M.D.

Satisfying Unmet Medical Needs

Dr. Ladner is particularly enthusiastic concerning Dyax' antibody phage library, which combines human and synthetic antibody sequences. The light chains and heavy-chain CDR3 variable regions of human origin are combined with additional synthetic sequences for heavy-chain CDR1 and CDR2.

The library's depth allows a vast number of different antibody heavy and light chain combinations to be sampled creating a wealth for lead selection, Dr. Ladner insists.

A typical protocol will result in the isolation of 300 or more antibodies. These candidates are then winnowed down

through the application of cell-based and other assays to eliminate unpromising molecules. "A typical screening allows isolation of sub nanomolar antibodies in the first selection," Dr. Ladner adds.

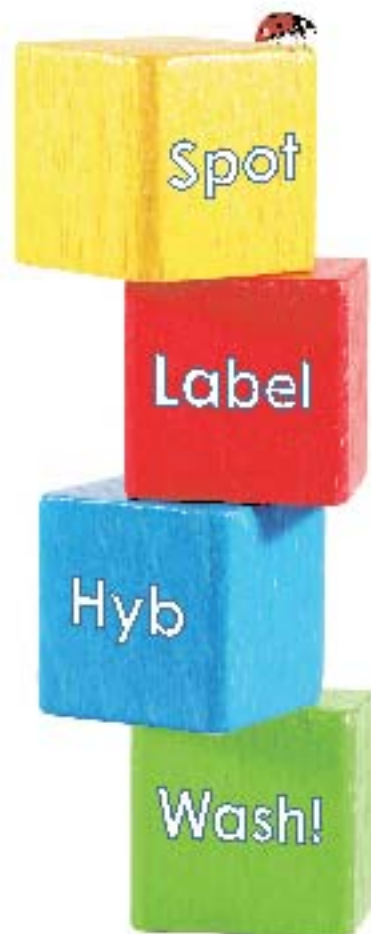
The success of epitope mapping in developing vaccines for infectious diseases has stimulated efforts in cancer vaccine research. There is great interest in cancer vaccines, and substantial resources and effort are being invested in their development.

Consensus on T-cell Immunity

Important advances have been made in the basic science of T-cell epitope function along with new commercial technology to put this knowledge to work in designing therapeutics. In order to consolidate these findings, Dr. de Groot and her colleagues have built a collective consensus leading to the publication of a white paper on T-cell immunogenicity screening for protein therapeutics (www.tcellwhitepaper.com).

Three workshops are scheduled to be held this spring to review methods for predicting and confirming T-cell immunogenicity (in silico, in vitro, and in vivo). New visions of immunogenicity and standards for its determination will be discussed. The goal is to delineate the contribution of cellular immune response to protein therapeutics and point to the frontiers of investigation, including the areas of regulatory T cells, tolerance, and cytotoxic T cells. **GEN**

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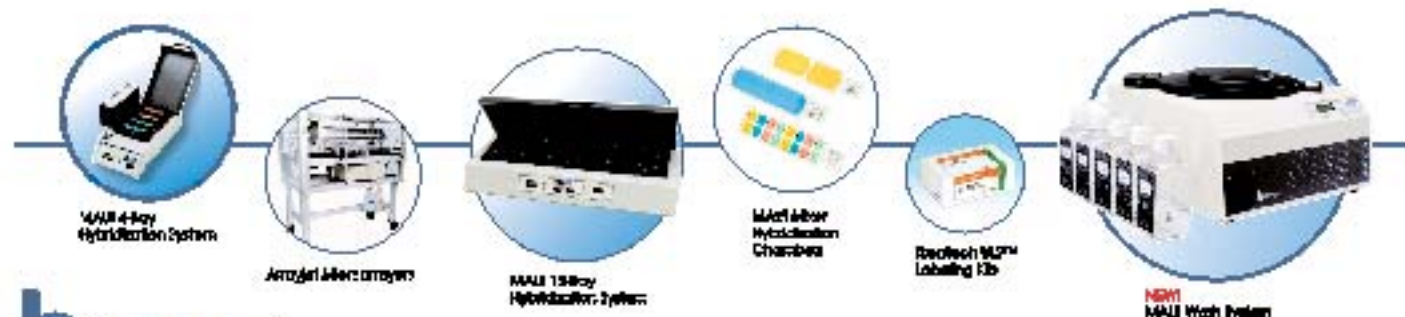
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