Peptides of fortune

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In the television show *Wheel of Fortune*, participants try to guess a phrase based on a few letters placed at specific positions in words of defined length. Precisely the same principle has now been applied by Bevan and colleagues on page 852 of this issue¹ to identify a specific peptide that allows protective cytotoxic (killer) T cells to identify and destroy cells infected with the intracellular bacterium *Listeria monocytogenes*. This finding and others open the way to the rapid identification of peptides that might be used to create synthetic vaccines; such vaccines would be invaluable in the fight against the many infectious diseases that continue to exact a heavy toll on human populations².

Immunological memory

Vaccination works by inducing a state of lasting immunity known as immunological memory. Memory requires the activation of T lymphocytes specific for the infectious agent. The T cells detect infection within cells by recognizing peptide fragments derived from the pathogen. These fragments are transported to the cell surface bound in a deep groove on the outer aspect of a molecule encoded in the major histocompatibility complex (MHC)^{3,4}. CD4⁺ T cells, which activate B cells and macrophages, recognize MHC class II molecules binding peptides of about 15 amino acids derived from proteins degraded in cellular vesicles. CD8⁺ T cells, which kill cells infected with viruses or intracellular bacteria such as *L. monocytogenes*, recognize MHC class I molecules binding peptides of eight or nine amino acids derived from proteins degraded by cytosolic proteasomes and transported into the endoplasmic reticulum by specialized transporter molecules of the ATP-binding cassette family⁵. Individual amino-acid side-chains, located at precise positions along the peptide, bind in deep pockets that line the peptide-binding groove of MHC class I molecules⁶. The position of these pockets and the amino acids that line them are different for each MHC class I allelic variant; thus, each MHC class I molecule binds a different set of peptides. This MHC polymorphism causes differences between individuals in resistance to specific infections⁶.

The stable binding of peptides to MHC class I molecules allows their direct characterization, and Van Bleek and Nathanson⁷ recently sequenced a single peptide of the influenza virus eluted from a specific MHC class I molecule isolated from infected cells.

The peptide was recognized by CD8⁺ killer T cells in mice of that genotype, demonstrating that peptides identified in this way are functionally relevant. This approach extended earlier studies of Rammensee and colleagues⁸, who from whole-cell lysates had isolated small peptides that could sensitize cells for killing by specific T cells. Rammensee's group subsequently showed that pooled peptides of eight or nine amino acids, eluted from a purified MHC class I molecule, had dominant amino-acid residues at one or more positions⁹; peptides from different MHC class I molecules yielded distinct amino-acid motifs. This result has been confirmed in a specific case by Jardetzky *et al.*¹⁰ by sequencing individual peptides isolated from the MHC class I molecule HLA-B27.

Bevan's group¹ used the sequence motif X-Y-X-X-X-X-X-X-(V,I,A,L) (single-letter code) for H-2K^d-associated peptides to identify a specific peptide of nine amino acids derived from *L. monocytogenes*. This peptide is derived from listeriolysin, the bacterial protein that enables *L. monocytogenes* to enter the cytoplasm. The CD8⁺ killer T cells were known to recognize a fragment of listeriolysin bonded to the mouse class I molecule H-2K^d. Having produced all 11 nonamers with this motif, they found that one efficiently sensitized H-2K^d target cells for lysis by their cloned T-cell line. A similar finding has also been reported by Rammensee and colleagues¹¹ who identified a nonamer from a synthetic peptide of 19 amino acids that had previously been shown to confer sensitivity on target cells. So knowing the peptide motif of a specific MHC class I molecule can allow rapid identification of specific peptide epitopes from antigenic proteins of known sequence.

How great is the simplification that this procedure allows? It is theoretically possible to make 5×10^{11} peptides of nine amino acids. Jardetzky *et al.*¹⁰, on the basis of 14 peptide sequences known to bind HLA-B27, can define a motif for that molecule that could accommodate over 1.3×10^7 peptides, indicative of the remarkable capability of any MHC molecule to present a wide range of distinct structures. This motif reduces the complexity of possible peptides by a factor of about 40,000. Nevertheless, the identification of one specific peptide is still a daunting task unless one has a fairly small target, such as the 19-amino-acid peptide studied by Rammensee and colleagues¹¹ or the challenging 59K product of the listeriolysin gene¹. So

although it is a marked improvement on random searching for peptides, the *Wheel of Fortune* approach still involves considerable work.

An alternative approach to identifying critical antigenic peptide epitopes, one which generalizes that employed by Van Bleek and Nathanson⁷, is suggested by the work of Jardetzky *et al.*¹⁰ for MHC class I and of my own group¹² for class II. The method involves purification of MHC molecules, isolation of the bound peptides, and separation of the peptides by high-performance liquid chromatography. Surprisingly, direct sequence analysis of several of the peaks identified single peptides which were derived from proteins abundant in the appropriate cellular compartment. Some of these were derived from an infectious agent, and the identified peptides were immunogenic when tested *in vivo*. This approach allows the identification of any abundant peptide, does not require the use of radiolabelled amino acids and, most important, does not require knowledge of either immune reactivity or the genome of the infectious agent. But it remains to be seen how well it will work with unknown infectious agents.

Clinical application

These new techniques, which were originally developed to answer basic questions about the nature of antigen presentation, are now ready for clinical application. They should speed the search not only for peptides for use in vaccines, but also for autoantigens¹³ important in clinical medicine. A combination of direct peptide sequencing and the use of specific motifs in known antigenic proteins should generate much new information about the involvement of such peptides in specific infectious and autoimmune diseases. □

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