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Research Article

Structural Basis of Antigen Recognition by T Lymphocytes

Implications for Vaccines

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T lymphocytes are essential to both classical arms of the immune system, the cellular and the humoral. Cytotoxic T cells are the main antigen-specific effector cell of the cellular immune system, destroying infected autologous cells before an intracellular pathogen such as a virus can replicate and spread, and probably functioning in immune surveillance to destroy transformed cells before a malignant tumor can be established. Helper T cells are the central regulatory cell of the immune system, and are necessary for the induction of cytotoxic T cells and for antibody production to protein antigens. In addition to their ability to help in primary and secondary or anamnestic (memory) antibody responses by B cells, and their facilitory role in cytotoxicity of other cells, helper T cells can act as effector cells, both in direct killing of tumor targets (1, 2) and via secretion of lymphokines such as gamma interferon. Despite their clear importance in disease, T cells have been relatively neglected in vaccine design and development compared with antibodies, perhaps because antibodies are easier to measure and to understand. T cell activation, on the other hand, is a much more complex process that is only now beginning to be understood. This new understanding has led to some insights into the types of antigenic structures recognized by T cells that are beginning to prove useful in the rational design and development of vaccines. What follows is a brief summary of these concepts and their experimental basis, with illustrations of their current and potential applications.

In comparison with antibody recognition of structures on the surface of native proteins free in solution (3, 4), T cell recognition is much more complex, in that the antigen is not seen free in solution, but only on another cell, only after it has been taken up by that other cell and metabolically degraded as discussed below, and then only after fragments of the antigen have associated with surface proteins encoded by the MHC of that other cell (5–7). This other cell has been called an antigen-presenting cell (APC)[1] because it "presents" antigen to T cells. In the case of helper T cells, this cell is most often a macrophage, dendritic cell, or B lymphocyte, although other cells can serve this function, whereas in the case of cytotoxic T cells, almost any nucleated cell can present antigen and be a target (7, 8). This difference has to do with the fact that most, but not all, helper T cells recognize antigen in association with class II MHC molecules, such as HLA-DP/DQ/DR in the human and I-A and I-E in the mouse, which are expressed only on certain cell types, whereas conventional cytotoxic T cells usually, but not always, recognize antigen associated with class I MHC antigens, such as HLA-A, B, and C in humans and H-2K and H-2D in the mouse, which are expressed on most cell types. Soluble antigens may be taken up by such nonspecific mechanisms as pinocytosis or phagocytosis, or by specific receptor-mediated endocytosis. In the latter category would be binding of antigen to B lymphocytes specific for that antigen by means of their specific surface immunoglobulin, and binding of antigen-antibody complexes to the Fc receptors of macrophages or B lymphocytes (9–14). Once in the cell, such exogenous antigen is usually proteolytically degraded in endosomes into fragments (7, 8, 15), although unfolding of the intact protein without actual proteolysis has been shown to be sufficient to allow presentation of antigen in a number of cases (16–18). The details of this processing event, which is probably needed to expose all of the relevant parts of the antigenic structure for binding simultaneously to the MHC molecule and to the T cell receptor, are not yet completely understood. The products of processing make their way back to the surface, probably via the Golgi (19). It is not yet clear whether the association with MHC molecules takes place in the endosome or on the cell surface, but such exogenous antigens usually associate with class II MHC molecules. In the case of cytotoxic T cell recognition of antigen with class I MHC molecules, the antigen is usually a protein synthesized in the presenting or target cell, such as a host cellular protein or a viral protein. These internally synthesized proteins are processed by a distinct pathway, probably in the cytoplasm, and tend to associate with class I MHC molecules (20–23), although exceptions exist (24). The discovery that cytotoxic T cells recognize processed antigen fragments rather than intact proteins, even in cases where the intact protein such as influenza hemagglutinin normally exists on the cell surface (20), has led to the realization that both types of T cell recognize antigen in a similar way, as proteolytic fragments associated with a MHC molecule. Thus, despite the differences in processing pathways and MHC molecules, the remainder of the recognition steps appear to be equivalent. Our understanding of the binding of antigen peptide fragments to MHC molecules has been greatly enhanced by the ability to do direct binding studies in the case of class II MHC molecules (25, 26) and by the x ray crystallographic determination of the three-dimensional structure of the class I human HLA-A2 molecule (27, 28). These events are reviewed in more detail in references 6 and 29–31.

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1. *Abbreviations used in this paper:* APC, antigen-presenting cell; *Ir*, immune-response.

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This complex process of antigen recognition by T cells leads to several constraints on T cell recognition. The simplest, but in some ways least understood, is the ability of T cells to recognize protein and peptide antigens, but with a few exceptions, not carbohydrates, in contrast to antibodies, which recognize both. Second, the recognition of antigen only when associated with a MHC molecule leads to the phenomenon of immune-response (*Ir*) gene control of responses to each antigen. *Ir* genes are genes that determine the ability of an individual to respond to a particular antigen, and in this antigen specificity differ from other genes, in which defects lead to broad hereditary immune deficiency diseases. Many of the antigen-specific *Ir* genes have been shown to correspond to the structural genes for the MHC antigens (6), and it appears that *Ir*-gene defects in the response to specific antigens are due to failure of peptide fragments of these antigens to bind to particular alleles of the appropriate MHC molecules, and to associated gaps in the T cell repertoire for recognition of particular peptides in combination with particular MHC molecules (6, 25, 32–35). Consistent with this idea that *Ir* gene control depends on the binding of individual peptide fragments of an antigen to MHC molecules, it was found that the response to different antigenic sites on the same protein antigen may be controlled by different *Ir* genes (36). Third, this complex process of recognition leads to the phenomenon called immunodominance, that is, the skewing of the T cell response to a protein antigen to primarily one or a few of the many potential antigenic sites on that protein. Immunodominance is therefore of interest for understanding problems ranging from autoimmune disease to vaccine design, and will be a major theme of this Perspectives article.

The complexity of T cell recognition of antigen ironically introduces one important simplifying feature. Because T cells recognize peptide fragments of proteins that have primary (amino acid sequence) and secondary structure (such as α-helices and β-turns) but largely do not retain the tertiary structure or folding of the native protein, it is much easier to study them with short synthetic peptides and to determine the critical structures for recognition. Many antibodies interact with structures assembled on the surface topography of the native protein from discontinuous segments of polypeptide (called "assembled topographic sites") (3, 4) and therefore require intact proteins with x ray crystallographic structures to understand antigen recognition on a molecular level (37–39). Computer modeling of proteins is not developed sufficiently yet to predict the tertiary folding of a protein from its amino acid sequence alone. Fortunately, for T cells this is not necessary. Primary and secondary structure are much more accessible to modeling with current techniques, and so it is ironic that it may be easier to predict structures recognized by T cells than to predict those recognized by antibodies, even though the chemistry of antibody recognition is better understood than that of T cell recognition.

Importance of immunodominance. The observations noted above that the T cell response to a protein antigen is a composite of the responses to individual peptide fragments of the protein, each under potentially different *Ir*-gene control, led to the concept that high responsiveness to a protein results from response to many more antigenic sites on the protein than are recognized by genetic low responders, and that, in the simplest view, the level of responsiveness is a function of the number of antigenic sites that can be presented by the MHC molecules of the individual responding (36). This simplistic accounting must now be modified in view of the finding that responses to some antigenic sites (the immunodominant ones) can be much greater in magnitude than the response to others (40–42). We therefore asked whether the *Ir* gene control of the response to a whole protein could be dominated by the *Ir*-gene control of the response to a single immunodominant site. Kojima et al. (43) did a limiting dilution measurement of the frequency of T cells specific for different antigenic sites of myoglobin, as a model protein antigen, in association with different MHC molecules, in F_1 hybrid mice from a cross between a high-responder MHC type and a low-responder MHC type. One site represented more than two-thirds of the T cells specific for myoglobin in association with the high-responder MHC molecules, but was absent among the repertoire of T cells specific for myoglobin with low responder MHC molecules. Thus, even though T cells of both MHC specificities responded similarly to other antigenic sites on the myoglobin molecule, the presence or absence of a response to a single immunodominant antigenic site was sufficient to account for high or low responsiveness. For this reason, immunodominant structures should be a critical focus for the design of vaccines. It is not sufficient to identify the antigenic site recognized by a single clone, unless one knows that that clone is representative of the dominant population.

Immunodominance depends on more than the ability to bind to a particular MHC molecule, since the same site of myoglobin, 102–118, was immunodominant in association with several MHC types (41, 44). Indeed, it is this immunodominance across many MHC types within a species, and even across species, that led us to consider other factors that might contribute to immunodominance, as discussed below. Immunodominance is also not simply a result of self tolerance to homologous structures on autologous proteins, as might be considered in the case of model proteins such as myoglobin or cytochrome *c*. Similar immunodominance has been observed with bacterial (45), viral (46, 47), and parasite-derived (48) proteins that bear no apparent homology with the host.

One property of immunodominant sites that may in part account for the large number of T cells that respond to them may be that they generally consist of a cluster of overlapping antigenic subsites seen differently by a number of distinct T cell clones. Thus, immunodominant sites are actually the focus of a large polyclonal response (49–51).

This finding, however, also means that immunodominance is not dependent on the response of any particular T cell clone, but must depend on other factors. These factors can be divided into those intrinsic to the structure of an antigenic site and those which are extrinsic to this structure and which depend on other features of the protein antigen or the responding host (42, 52).

Role of peptide binding to MHC molecules. The correlation between epitope specificity of T cells and the MHC molecule on the APC presenting the antigen (reviewed in references 5, 6, 53, and 54) led to the concept that *Ir*-gene control was due to the binding of different peptides to different MHC molecules (32, 33). Studies using related peptides to compete for antigen presentation suggested specific binding to MHC molecules (55, 56), and the ability of unrelated peptides to compete suggested that there was only a single site on the MHC molecule for binding peptides (57). However, it has been only recently that such binding has been directly demonstrated using purified class II MHC molecules (25, 26). Moreover, the correlation between the ability of a peptide to bind, the ability to

compete, and the ability to activate T cells in association with a particular MHC molecule strongly suggests that the specificity of peptide binding to MHC molecules is indeed a major mechanism of *Ir*-gene control (34). The analysis of the structural basis of this specificity has been greatly facilitated by the recent x ray crystallographic determination of the structure of one of the receptors, the class I human HLA-A2 molecule (27, 28), and the hypothetical modeling based on this of the class II MHC molecule (58). The combining site of the MHC molecule for peptide appears to be a groove ~ 10 Å wide formed by two α-helices, one from each of the two outer domains of the protein, running roughly antiparallel to each other, with a floor beneath consisting of β sheet. This groove would nicely accommodate another α-helix of peptide. The residues pointing into the groove include many of the polymorphic residues within the MHC molecule and could account for the differences in fine specificity of different MHC molecules. The use of recombinant MHC genes has recently allowed the mapping of distinct residues on the same MHC molecule that bind to peptide and that bind directly to the T cell receptor (59). Preliminary attempts have been made to find primary sequence patterns in peptides binding to the same MHC molecule that could account for the specificity of binding (35, 60, 61), but at present the patterns are not sufficiently well defined to use predictively. It should be pointed out that the selection of epitopes by MHC molecules is just that, a selection of a subset that can be seen by a given individual from a repertoire of structures that would make good potential T cell antigenic sites in general. Thus, this selection operates at a different level from the intrinsic structural features of immunodominant sites to be discussed below.

Role of antigen processing. Although antigen processing may frequently involve proteolysis (15, 62), the finding that unfolding the antigen is sufficient to bypass the need for processing (16, 17) led to the concept that the purpose of processing is to expose amino acid residues that are not exposed in the native conformation of the protein. Even in a small peptide, if certain amino acids are unavailable because of disulfide bonds, the peptide may need processing (63), whereas a very large protein of > 300,000 kD may not need processing if the relevant residues are already exposed (64). Presumably, some residues need to be exposed to interact with the MHC molecule, whereas others need to be accessible simultaneously to the T cell receptor (65).

Nevertheless, processing that is sufficient for presentation by one MHC molecule may not be sufficient for presentation by another. For instance, in the case of the model protein antigen equine myoglobin, T cells from mice of one MHC type immunized with whole myoglobin respond primarily to one immunodominant site, residues 102–118, whereas T cells from mice of a different MHC type, immunized with the same myoglobin, do not recognize this site. Yet, when immunized with the synthetic peptide corresponding to this antigenic site, T cells of both MHC types can respond (66). Therefore, the failure to respond when immunized with the whole protein cannot be due to an inability of the antigenic site itself to bind to either MHC molecule, or to a lack of T cells in the repertoire that can see this site. The difference between the results with native protein and short synthetic peptide must be related to the processing of the native molecule, because the difference can be overcome by artificial cleavage to produce large fragments of the protein. Studies with F_1 hybrid APCs demonstrated that the difference was not due to differences in the mode of processing between APCs of the two MHC types (66). The best explanation was that the natural product of processing was larger than the synthetic peptide corresponding to this antigenic site, and contained structures that still hindered interaction with one MHC molecule but not another (66). Similar findings were made for a site on hen lysozyme (67).

The concept of hindering structures on the products of natural processing can also explain the observations with both myoglobin (66) and lysozyme (68) that T cells that readily distinguish between two homologous proteins from different species may fail to discriminate at all when given the short peptides corresponding to the antigenic sites alone. The difference between the homologous proteins must be outside the sequence of the antigenic site itself. This idea is also consistent with the observation that the conformational state of the whole protein can influence T cell recognition (69). Furthermore, even short synthetic peptides that ordinarily do not need processing may be processed in a way that affects specificity or MHC interaction (70). All of these results together indicate that processing must be considered another important level at which *Ir*-gene control and immunodominance are regulated. For these reasons, the proteases involved in processing are being identified to try to predict the location of cleavage sites within proteins (71; and Takahashi, H., et al., manuscript submitted for publication). From a practical point of view, the finding of hindering structures on the products of processing indicates that it is important to be sure not simply that a peptide is immunogenic for T cells, i.e., elicits T cells that respond to the peptide itself, but also that the peptide elicits T cells that can cross-react with the whole protein (that is, the products produced when the whole protein is processed). Otherwise, the peptide will not be of much value in a vaccine.

Intrinsic structural features of T cell antigenic sites. Although each MHC molecule may select a subset of potential T cell antigenic sites, it would be of value to know whether the total potential repertoire consists of every segment of the protein equally, or whether there are structural features that make certain peptide segments more likely to be immunodominant than other segments in the same protein. Knowledge of such features would greatly facilitate the search for immunodominant sites and the design of synthetic vaccines. The fact that T cells generally see short peptides implies that only primary structure (amino acid sequence) and secondary structure (such as helices and bends) that depends on local interactions need be considered. As discussed earlier, this simplifies the search for such features in T cell antigenic sites compared with those in antibody sites (3, 4, 18, 72).

One such structural feature appears to be α-helicity, first noted for the case of the immunodominant T cell antigenic site of pigeon cytochrome *c* (73, 74) and then generalized in a statistical analysis of 23 immunodominant sites from 12 proteins (75). The explanation for this preference for helices may lie in their inherent structural stability in peptides in the right environment (75), or in the shape of the MHC peptide-binding groove describe above that has the right dimensions to accommodate an α-helix (27, 28, 58). Also, a statistical tendency to have lysine residues near the carboxyl terminus of the site (75) may in part relate to the stabilizing effect on a helix, but appears to be statistically independent of helicity and so may be an additional structural feature of T cell sites (75).

A related but independent structural feature is helical amphipathicity, that is the property of a helix having one side hydrophobic and the other side hydrophilic. The possibility

that such amphipathic structures might be favored as immunodominant T cell antigenic sites was proposed by DeLisi and Berzofsky (76) on the basis of theoretical considerations and also examples from the model protein myoglobin in which the immunodominant sites were amphipathic helices in which, in one case, both the hydrophobic and hydrophilic sides were found to be necessary (16, 77). Their analysis of 12 sites from 6 proteins was extended to 23 sites from 12 proteins by Margalit et al. (78), who developed an optimized computer algorithm called AMPHI that searches protein sequences for such structures. 18 of the 23 sites (~ 75%) were predicted ($P < 0.001$). Amphipathicity was found to be important statistically, independently of helicity per se (75). As the database of known immunodominant sites seen by helper-type T cells in association with class II MHC molecules (human HLA-DR/DQ/DP, mouse I-A, I-E) has more than doubled to 48 such sites found without the use of predictive algorithms that might bias the analysis, the correlation has held up. 34 (~ 71%) are still predicted and the correlation is still highly significant relative to other segments in the same set of proteins ($P < 0.003$) (78a). This suggests that there is something about the amphipathic helices that makes them favored to be immunodominant T cell sites. Some of these sites are known not to be helices in the native protein from which they derive, but since the T cell does not see the native protein, but only a small peptide fragment after processing, what may be important is not the structure in the native protein but a sequence that can fold into an optimum structure when freed of the constraints of the whole protein. However, it is clear from the fact that nearly 30% of immunodominant sites do not have this property that there are other structures that can serve as immunodominant T cell sites. To date, we have not been able to discern a common structural feature of these nonamphipathic sites. Also, most synthetic peptides, when used to immunize, can elicit some T cell response to themselves, so the preference for amphipathic helices may apply only to sites that are immunodominant relative to other parts of the same protein. Since it is the immunodominant sites that should be most useful in a vaccine, this predictive approach may be useful for searching for such sites for vaccine development (79).

Although originally there were too few antigenic sites recognized by cytotoxic T cells in association with class I MHC molecules (human HLA-A/B/C, mouse H-2K/D) defined with peptides to analyze in this way, the discovery by Townsend et al. (80) that short synthetic peptides can sensitize targets for cytotoxic T cells has opened the door to identifying such sites. With 11 such sites now defined, 10 are predicted by the AMPHI program (78a). Of these, four were located by predictive algorithms that might bias the results, but of the remaining seven discovered in an unbiased way, all seven are predicted by AMPHI. Thus, although such a high percentage is unlikely to hold up as the sample size grows, it appears that the same type of structure is seen by cytotoxic T cells in association with class I MHC molecules as is seen by helper T cells in association with class II MHC molecules. From this result, we would guess that both types of T cells, or both types of MHC molecules, select from the same pool of potential sites, so that the difference in repertoire between a class I MHC and a class II MHC molecule should not necessarily be greater than the difference in repertoire between class II MHC molecules of two different isotypes (e.g., DR vs. DQ). As cytotoxic T cells are an important effector mechanism in immune defense, the ability to predict sites immunodominant for cytotoxic T cells should be equally valuable in vaccine development.

Other properties have also been correlated with T cell antigenic sites. Stille et al. (81) described a strip-of-the-helix algorithm that searches for amphipathic helices, but does so somewhat differently, focusing more on the hydrophobic side of the helix. Rothbard and Taylor (61) took a more empiric approach, searching for amino acid sequence patterns common to T cell antigenic sites (both those seen by helper and those seen by cytotoxic T cells). They described a pattern of four or five residues consisting of a charged amino acid or glycine followed by either two or three hydrophobic residues followed by a hydrophilic amino acid. For this purpose, threonine and tyrosine were allowed to be hydrophobic or hydrophilic as necessary. This pattern or motif was found to be present in a large fraction of T cell antigenic sites, and was used successfully prospectively to locate new antigenic sites in several proteins from influenza virus (82) and mycobacteria (83). However, the structural basis of this sequence motif is not understood. One possibility is that it correlates with amphipathic helices, since the motif corresponds to one turn of an amphipathic helix.

We have used the amphipathicity algorithm to prospectively locate immunodominant helper T cell epitopes from the circumsporozoite protein of *Plasmodium falciparum* malaria (84), a protein from the blood stage of malaria (85), the AIDS viral envelope protein (86), and the acetylcholine receptor for T cells of patients with myasthenia gravis (87). Also, when peripheral blood T cells from humans living in a malaria-endemic area of West Africa were studied with a panel of overlapping synthetic peptides covering the whole circumsporozoite protein, four major T cell sites were identified, all falling into regions predicted on the basis of helical amphipathicity (48). We have now also studied a large set of overlapping peptides covering about half of the HIV envelope protein gp160, and have found additional sites, with a strong tendency for the immunodominant sites to be in amphipathic helical regions of the sequence (Hale, P. M., et al., manuscript submitted for publication). For both malaria (48, 84) and the HIV envelope protein (86, 88), the same sites that were seen by mouse T cells were seen by human T cells as well, suggesting that the same principles apply to the selection of immunodominant antigenic sites in both species, and that studies in this animal model may be directly applicable to vaccines aimed at human disease.

In conclusion, antigenic sites that are immunodominant can command a sufficient part of the T cell response to make the difference between high and low responsiveness to a protein antigen. Certain structural features intrinsic to short peptide sequences, such as α-helicity, helical amphipathicity, and sequence motifs consistent with these structures, tend to make certain parts of protein sequences more likely to be immunodominant sites for recognition by both helper and cytotoxic T lymphocytes, although other structures not fitting these patterns can be immunodominant as well. From this repertoire of potential sites within a protein, other factors determine which sites are seen by T cells from any given individual. These include the ability to bind to the MHC molecules of that individual, and the way the antigen is processed by antigen presenting cells into fragments before T cell recognition. There has been some concern that peptide antigenic sites may not be useful as vaccines because any single antigenic site will be seen by only a subset of individuals. However, the discovery of

sequences such as residues 428–443 of the HIV envelope that can be seen by T cells from multiple MHC types of mice (86; and Hale et al., manuscript submitted for publication), goats (Palker, T., et al., manuscript in preparation), chimpanzees (Krohn, K., et al., manuscript submitted for publication), and humans (88) suggests that peptides may be found to overcome this problem.

In cases such as AIDS, in which an attenuated live virus or even killed whole virus vaccine may not be acceptable for safety reasons, a highly engineered artificial vaccine may have some advantages over a vaccine consisting of individual natural subunits (79, 89). If one can localize immunodominant helper and cytotoxic T cell antigenic sites and sites that induce neutralizing antibodies, one can construct a vaccine by either solid-phase peptide synthesis or recombinant DNA techniques that incorporates these relevant sites, and excludes sites that might induce suppressor T cells or enhancing antibodies that actually facilitate viral uptake. Further, one can incorporate several variants of important sequences if these derive from variable parts of the antigenic protein, to cover more isolates or strains of the pathogen. In addition, one can produce multiple repeats of the same antigenic site to attempt to increase immunogenicity beyond that achieved with the natural protein antigen. Because pathogen proteins have evolved to avoid immune recognition rather than to optimize it, it is logical that an artificial construct of the sort proposed here may actually be able to improve on nature, at least in the limited situation in which one's goal is different from the results of evolution. Although the field of completely artificial vaccines is new and there is little experience in the most effective ways to construct them, it is hoped that the basic studies outlined in this Perspectives article will contribute to the effort to rationally design such a new generation of vaccines.

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