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Structural Features of T-Cell Recognition: Applications to Vaccine Design

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Structural features of T-cell recognition: applications to vaccine design

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T lymphocytes, in contrast to antibodies, appear to recognize primarily a limited number of antigenic sites on any given antigenic protein. We find that a single site can so dominate the T-cell repertoire that the presence or absence of a response to one immunodominant site can make the difference between a high responder and a low responder, even though low responders respond to other sites almost as well as high responders. Besides interaction with major histocompatibility complex (MHC) molecules, the mode by which the antigen is processed into fragments for T-cell recognition also determines which sites are seen. The products of natural processing of the protein appear to be larger than the synthetic peptides and contain structures which hinder binding to certain MHC molecules or to the T-cell receptor. A third factor in immunodominance is the intrinsic structure of the antigenic site. We have shown that amphipathic helices have a higher than random chance of being immunodominant, and have developed a computer program to locate such structures in protein amino acid sequences. We prospectively predicted sites in the malaria circumsporozoite protein and found that the four most widely recognized sites in an endemic area of West Africa were all predicted. Similarly, we identified two helper T-cell sites from the HIV (AIDS virus) envelope, and have now shown that immunization with these elicits enhanced antibody responses to the whole envelope when injected into monkeys. These sites are also recognized by human T cells from volunteers who had been immunized with a recombinant vaccinia virus expressing the HIV envelope. Also, because cytotoxic T lymphocytes (CTLs) may play a critical role in defence against AIDS, we have used a recombinant vaccinia virus and transfectants expressing the HIV envelope gene to induce specific CTLs against the HIV envelope. Using synthetic peptides, we were able to identify the first CTL recognition site in the AIDS virus. These results may contribute to the rational design of vaccines.

Introduction

In contrast to antibodies that bind antigen free in solution and often only in the native conformation (Benjamin *et al.* 1984; Berzofsky 1985*a*), T cells recognize antigen only on the surface of another cell, and, for native proteins, only after they have been 'processed' by proteolysis or at least unfolding (Unanue 1984; Berzofsky 1985*b*, 1987; Allen 1987). Resulting processed fragments associate with molecules encoded by the Major Histocompatibility Complex (MHC) of the cell 'presenting' the antigen to the T cell, and the T-cell receptor appears to recognize this complex (Benacerraf 1978; Rosenthal 1978; Babbitt *et al.* 1985; Buus *et al.* 1987). This complex mechanism leads to the immunodominance of a few antigenic sites in each protein (Berzofsky 1986, 1988). We have observed that immunodominance depends on the mode of processing and intrinsic features of the site as well as binding to the MHC molecule. An understanding of the principles that lead to immunodominance may contribute to the rational design of vaccines, in which these immunodominant T-cell antigenic sites should play a major role.

The importance of immunodominance

The structural genes of the MHC have been determined to be the functional genes described originally as Immune Response (*Ir*) genes on the basis of their ability to control the level of immune response to individual antigens (reviewed in Berzofsky 1987). In the case of multi-epitope protein antigens, high responders were found to respond to more epitopes than intermediate or low responders, at both the antibody and T-cell level, and the responses to different epitopes on the same protein were controlled by potentially distinct *Ir* genes (Berzofsky *et al.* 1979; Kohno & Berzofsky 1982). This led to the notion that level of responsiveness was in part related to the number of epitopes recognized. However, this concept needs to be modified in that not all epitopes carry equal weight in this equation. We have recently found by a limiting dilution analysis of the frequency of T-cell clones specific for different epitopes of myoglobin, that the overall three-to-fourfold difference in the total frequency of clones responding to myoglobin in association with high- or low-responder MHC types can be accounted for largely by the frequency of clones specific for one single epitope (Kojima *et al.* 1988). This epitope, comprising residues 102–118, appears to be the major immunodominant site in two high-responder haplotypes, H-2^d and H-2^s, and to be unrecognized by two low-responder haplotypes, H-2^k and H-2^b (Berkower *et al.* 1982, 1984, 1985; Kojima *et al.* 1988). In this study, high responders and low responders were found to have nearly equal frequencies of clones responding to other regions of the molecule, such as sites in the 1–55 fragment, but in the low responders, these constitute the bulk of the response. The responses to these other sites never compensate for the lack of responsiveness to the immunodominant site 102–118. Thus the presence or absence of the response to a single site can make the difference between high and low responsiveness. We also conclude that there must be something special about this immunodominant site.

In studying the specificity of T-cell clones for this site, we found that different clones see it in slightly different ways, as assessed by their responses to overlapping and nested series of peptides (Cease *et al.* 1986*a*). Similar conclusions were reached by E. E. Sercarz's laboratory (Manca *et al.* 1984; Shastri *et al.* 1985) and by Allen *et al.* (1985) for lysozyme, and similar observations were made for ovalbumin (Shimonkevitz *et al.* 1984) and cytochrome *c* (Schwartz *et al.* 1985). On the one hand, we may hypothesize from these results that this focusing of a multiplicity of clones on a single site contributes to the frequency of clones specific for that site and therefore its immunodominance, although this does not explain the reason for this focusing. Also, we do not yet know whether similar focusing occurs for non-immunodominant sites. On the other hand, this result also implies that immunodominance is not caused by the pre-emption of the response by a single clone of T cells, and, indeed, is independent of any single clone of T cells responding. We must therefore look outside the T cell for the reasons for immunodominance of a given site.

In principle, we can divide the factors that determine immunodominance into those intrinsic to the structure of the antigenic site itself, and those extrinsic to the antigenic site, such as factors in the host making the immune response.

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Extrinsic factors in immunodominance

The requirement for binding of antigenic peptide to the MHC molecule for T-cell recognition clearly plays a major role in selecting which sites will be immunodominant in any given MHC haplotype (Rosenthal 1978; Benacerraf 1978; Berzofsky 1986, 1988). This binding has been studied extensively (Babbitt *et al.* 1985; Buus *et al.* 1987; reviewed in Berzofsky 1987, 1988). In the case of myoglobin, we observed that one site is immunodominant with I-A^d, but that if I-E^d is the only presenting element available, then another site, 132–146, is immunodominant (Berkower *et al.* 1985; Kojima *et al.* 1988). The major competing hypotheses for the mechanism of *Ir*-gene control are, indeed, the ability of the relevant peptide to bind to the MHC molecule of the animal in question, and the presence of T cells with the appropriate specificity in the repertoire of that animal (Berzofsky 1987).

However, we have observed that other factors such as antigen processing can play a major role in the determination of immunodominance and therefore *Ir*-gene control. For example, H-2^k mice fail to produce T cells responding to the 102–118 site of equine myoglobin when immunized with the whole molecule, and yet when immunized with the synthetic 102–118 peptide, they produce T cells responding perfectly well to 102–118 in association with I-A^k (Brett *et al.* 1988). The antigenic site 102–118 can therefore bind to I-A^k, and there are T cells in the repertoire of these mice that can see that site. Thus neither of the conventional mechanisms of *Ir*-gene defects appears to be able to explain these results. A clue comes from the fact that the T cells elicited by immunization with the peptide fail to cross react with the native protein. It therefore appears that the natural product of processing of the native protein and the synthetic peptide do not cross react in this strain of mice, even though they do in mice of another MHC haplotype (H-2^s). As the strains of mice differ only in MHC, one would imagine that the same peptide fragments are produced by processing in both. To test for MHC-linked processing differences as the cause, we took advantage of the fact that we had T-cell clones of both haplotypes that saw essentially the same epitope. F_1 hybrid-presenting cells that could process and present the native protein to H-2^s T cells specific for 102–118 must be able to produce a fragment containing the epitope. However, the same F_1-presenting cells failed to present this epitope to H-2^k T cells when the native antigen was used. Thus the results are not due to an H-2-linked failure to produce a fragment containing the appropriate epitope, but rather to production of a fragment in presenting cells of either MHC type or the F_1 that can interact with I-A^s but not I-A^k. Also, as we see the same result with single T-cell clones the results cannot be due to the presence of suppressor T cells that react with other sites on the whole protein. We therefore presume that the product of natural processing of myoglobin is larger than the synthetic 102–118 peptide, and includes structures that hinder binding to I-A^k but not to I-A^s (Brett *et al.* 1988).

A similar conclusion can also be drawn from the observation that a T-cell clone that responds to sperm whale but not equine myoglobin when the native proteins are used responds to the synthetic peptides 102–118 from both species (Brett *et al.* 1988). Here it appears that the product of natural processing of equine myoglobin cannot be seen by this clone, whereas those of sperm whale myoglobin can, even though the antigenic site is present on both and can be seen equally well if the appropriate synthetic peptide is made. Similar results were found for lysozyme by Gammon *et al.* (1987). We conclude from all these studies that processing can play a very significant role in determining which antigenic sites are immunodominant. Antigenic

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sites, which alone could bind to the appropriate MHC molecules and be seen by appropriate T-cell receptors, may fail to be recognized because of structures outside the actual antigenic site but associated with it by the mechanisms of processing.

Intrinsic factors in immunodominance

Extrinsic factors discussed above, such as MHC interaction and processing, as well as others such as self-tolerance to homologous self-protein epitopes, all select from a repertoire of potential immunodominant antigenic sites. Therefore, one can ask, are there features intrinsic to the structure of these antigenic sites that favour them to be immunodominant, independent of the extrinsic factors that vary from host to host? If there are such factors, these would operate at a different level, on which the extrinsic factors would then be superimposed.

For myoglobin, we noted that both of the immunodominant sites 102–118 and 132–146 were amphipathic helices in the native protein, i.e. helices with one side hydrophobic and one side hydrophilic (Berkower *et al.* 1986; Cease *et al.* 1986*a*). Only about half of the α-helices in native proteins in the crystal structure database are amphipathic (Cornette *et al.* 1987). This property seemed potentially important because we could locate critical antigenic residues on the hydrophilic side of the helix (Berkower *et al.* 1985, 1986; Cease *et al.* 1986*a*), and yet our studies of antigen processing suggested that the purpose of processing was to unfold the molecule to expose residues that are not normally exposed in the native protein (Streicher *et al.* 1984; Berzofsky 1985*b*). In these latter studies, it was found that an unfolded form of the intact protein molecule behaved like the short peptide and did not need processing. A similar result was found for denatured lysozyme (Allen & Unanue 1984). What these unfolded structures have in common is that they expose residues on the hydrophobic side of the helix that are not exposed in the native molecule. We hypothesized that exposure of these hydrophobic residues may be important for interaction with some structure on the surface of the antigen-presenting cell, such as the MHC molecule or the membrane itself. If both the hydrophobic and hydrophilic side were important, each could serve a different function, such as binding to the T-cell receptor and to the MHC molecule. A similar model with two subsites for these two functions had been proposed for cytochrome *c* (Heber-Katz *et al.* 1983), but in that case the sites were thought to be separated along the linear sequence of the molecule, rather than interdigitated but separated in space by the helical folding as for the myoglobin amphipathic helices. We therefore hypothesized that this type of amphipathic helical structure might be a favoured structure for T-cell recognition (DeLisi & Berzofsky 1985). This idea is also consistent with the notion that because the T cell sees only short peptides cleaved from the protein, all the necessary information has to be in local primary and secondary structure, and not in tertiary structure.

To see whether this concept might be applicable to many proteins for which immunodominant T-cell epitopes had been identified, we developed a computer algorithm to look for such structures in protein sequences (De Lisi & Berzofsky 1985; Margalit *et al.* 1987). This algorithm is based on the idea that for a helix to be amphipathic, every time one moves full turn from a hydrophilic residue one wants to come to another hydrophilic residue, and similarly, a hydrophobic residue should be one full turn from a hydrophobic residue. Therefore, the hydrophobicity (where hydrophilic is the negative of hydrophobic) of the amino acids along the sequence should oscillate with a periodicity similar to the structural periodicity

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of the helix. Accordingly, the algorithm searches for regions of sequence with a periodicity of hydrophobicity such that if the sequence were folded as a helix, the helix would be amphipathic. A moving window of overlapping 11-residue segments, shifted just one residue at a time along the sequence, is tested for best-fit frequency of a sinusoidal function by a Fourier transform or least-squares best fit. For short sequences such as this 11-residue window, we have found that the least-squares method works best (Cornette *et al.* 1987; Margalit *et al.* 1987). By this approach, we analysed the sequences of 12 proteins for which 23 immunodominant class II MHC-restricted T-cell antigenic sites had been defined in the literature. Eighteen of the 23 were found to fall in regions of the protein sequence which, if folded as an α- or 3_{10}-helix, would be amphipathic (Margalit *et al.* 1987). This correlation, compared with the rest of the sequences of the same proteins, was highly significant ($p < 0.001$). The analysis has now been extended to a larger database of 48 sites, and the correlation remains significant (about 71% have such structures; $p < 0.003$). Although only a limited number of sites seen with class I MHC molecules have yet been defined (Townsend *et al.* 1986; Maryanski *et al.* 1986; Gotch *et al.* 1987), most also have this property. However, the database of class I-restricted sites is too small to determine statistical significance. It should be noted that there are clearly other ways of making immunodominant T-cell antigenic sites, as 25–30% of the known sites would not fold to make amphipathic helices. Nevertheless, this seems to be a favoured structure, and so may be a clue to the chemistry of the interaction as well as of practical importance in vaccine development. It should also be noted that not all of these sequences correspond to helices in the native proteins, in some of the cases where crystal structures are available. However, because the T cell does not see the native protein, but only a short peptide cut from the constraints of the native molecule, what matters may be the ability of that peptide to fold appropriately when placed in the right environment. An anisotropic environment, which has a hydrophobic and hydrophilic region, would stabilize a helix if that helix were amphipathic, because the helix would allow the hydrophobic residues to interact with the hydrophobic region of the environment and the hydrophilic residues with the hydrophilic regions. Indeed, peptides that have this periodicity were found to take on significant helical structure in the membrane mimetic environment of detergent micelles, as determined by circular dichroism (Sanza *et al.* 1987).

Another type of statistical analysis of immunodominant T-cell antigenic sites, using Monte Carlo computer techniques, has also indicated that amphipathic helices occur more frequently than by chance in the database of proteins with known immunodominant T-cell epitopes (Spouge *et al.* 1987, 1989). Indeed, α-helices themselves also occur more frequently than by chance alone. Helical tendency was found to be important for one particular epitope of cytochrome *c* studied in depth (Pincus *et al.* 1983; Schwartz 1985). Matching techniques demonstrate that these two significant correlations are independently significant; that is, neither one is significant merely because it is correlated with the other. In addition, a surprisingly high frequency of lysine residues near the carboxy terminus was found. These were more significant than could be accounted for simply by their helix-stabilizing tendency. Moreover, the random coil conformation was anticorrelated; i.e. lack of such coil tendency in the Garnier *et al.* (1978) parameters was significant. No significant association with β-conformations or β-amphipathicity was found.

Several possible explanations have been considered for the enhanced probability of amphipathic helical segments to be immunodominant T-cell antigenic sites. One is that helices

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fit snugly into the groove at the surface of the MHC molecule, based on the recent crystal structure of a class I MHC molecule (Bjorkman *et al.* 1987*a*, *b*) and the predicted model structure of a class II molecule (Brown *et al.* 1988). Moreover, the floor of the groove, although not completely hydrophobic, is more hydrophobic than the sides of the groove. Therefore, the amphipathicity may serve to orient the peptide helix rotationally in the groove, ensuring that the hydrophilic side is exposed to the T-cell receptor. Another possibility is based on the fact that amphipathic helices are known to be able to intercalate into membranes (Kaiser & Kézdy 1984). Because of the low affinity of peptide for MHC (Babbitt *et al.* 1985; Buus *et al.* 1986), we suggest that a peptide that can associate with the membrane for a longer period of time, and accumulate at a higher concentration, would have a greater chance of binding to the MHC molecule (Cease *et al.* 1986*b*; Berzofsky *et al.* 1987). Indeed, this idea led to the suggestion that the presenting cell membrane may serve as a reservoir or short-term memory of peptides from proteins recently encountered. These would be constantly sampled by MHC molecules so that if the appropriate T cell came along, it would bind, stabilize the complex, and itself be activated (Berzofsky *et al.* 1986, 1987). This idea has also been proposed by Falo *et al.* (1987). A third possibility is that amphipathic helices tend to be in less protruding portions of native proteins and so are less susceptible to proteases involved in processing. Regions that survive processing may be more likely to be available for T-cell recognition (O. Werdelin and J. Rothbard, personal communications).

A sequence pattern found by Rothbard & Taylor (1988) to be associated with T-cell antigenic sites for both class I and class II MHC molecule-restricted T cells, interestingly is consistent with one turn of an amphipathic helix. This finding may therefore have a mechanism in common. Also, another approach for searching protein sequences for amphipathic helices has been reported by Stille *et al.* (1987).

Practical applications to vaccines

Whatever the mechanism by which amphipathic helices seem to be favoured as immunodominant T-cell antigenic sites, this correlation may have practical application to vaccine development. As helper T cells are central to both antibody and cytotoxic T-cell immunity, and can secrete protective lymphokines such as interferon γ themselves, it is obviously important for a vaccine to contain epitopes that will be immunodominant for helper T-cell recognition. This amphipathicity approach may allow us to find such sites more efficiently than by large-scale screening.

Prospectively, we have predicted successfully four helper T-cell sites of the malaria *Plasmodium falciparum* circumsporozoite protein recognized by both murine and human T cells (Good *et al.* 1987, 1988*a*, *b*), and have coupled one of these to the major neutralizing antibody epitope to produce a synthetic construct that elicits immunity in mice that do not respond to the antibody epitope alone (Good *et al.* 1987). This type of construct, containing both a helper T-cell epitope and an antibody epitope from the same protein, may be useful in priming both T- and B-cell memory to allow an anamnestic response when the recipient is exposed to the pathogen in nature, as opposed to use of a foreign carrier protein that would not elicit T-cell memory for the pathogen. The four most widely recognized T-cell sites of the *P. falciparum* circumsporozoite protein in an endemic area of West Africa were all predicted, and only one strongly predicted site was not seen by this population (Good *et al.* 1988*a*).

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We have also identified prospectively three proliferative T-cell sites of the envelope of the human immunodeficiency virus (HIV) recognized by murine T cells, one of which can be used to immunize mice to elicit T cells that respond to the native envelope protein (Cease *et al.* 1987; Hale *et al.* 1989). Immunization with these peptides elicits enhanced antibody responses to the whole envelope when injected into monkeys (Hosmalin *et al.* 1989). Two of these sites tested are also recognized by human T cells from volunteers who had been immunized with a recombinant vaccinia virus expressing the HIV envelope (Berzofsky *et al.* 1988). Indeed, one of the sites (env 428–443) was seen by T cells from eight of 11 outbred HLA-diverse individuals that had been boosted 44 days before the test. Significantly for vaccine development, we observed in this study that those human volunteers who were boosted with antigen–antibody complexes of soluble protein fragment from the HIV envelope bound to a monoclonal antibody gave significantly higher proliferative T-cell responses than those who were boosted with soluble protein fragment alone (Berzofsky *et al.* 1988). We have also identified one immunodominant site for murine cytotoxic T-cell recognition of the HIV envelope (Takahashi *et al.* 1988), using a recombinant vaccinia virus and transfectants expressing the HIV envelope gene to induce specific CTLs against the HIV envelope in mice. However, we observed that such sites are very limited, and of five different class I MHC molecules in two MHC haplotypes that could have presented HIV envelope sites, only one appeared to be used (Takahashi *et al.* 1988).

Other malaria sites from the blood stage (Kabilan *et al.* 1988), as well as sites on the acetylcholine receptor recognized by peripheral blood T cells from patients with myasthenia gravis (Hohlfeld *et al.* 1988) have also been identified prospectively by the amphipathicity algorithm. Identification of such sites and construction of synthetic molecules containing helper T-cell, cytotoxic T-cell and antibody epitopes may help in the rational design of synthetic or recombinant vaccines. The hope is to improve on natural antigens in vaccines by making molecules that are more immunogenic and that exclude sites that might induce suppressor cells or that might lead to unwanted immune responses, such as enhancing antibodies that might actually increase viral uptake.

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