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T-cell antigenic sites tend to be amphipathic structures

(hydrophobicity/α-helix/antigen processing/protein secondary structure/myoglobin)

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ABSTRACT **We propose, on the basis of physical chemical and biological requirements for T-cell activation by antigen, that sites on a protein that can stimulate T lymphocytes will be capable of forming a stable amphipathic structure (i.e., one with separated hydrophobic and hydrophilic surfaces), displaying periodicity in hydrophobic residues. A spectral analysis of the 12 antigenic sites to which the method could be applied indicates that the amphipathic periodicity hypothesis is valid for 10 of them, generally with reliabilities that are well above 98%, with periodicities compatible with an α-helical structure. An 11th case manifests a different type of amphipathicity. The analyses require only a knowledge of amino acid sequence. The finding that T-cell antigenic sites show a high correlation with amphipathicity greatly simplifies the search for such sites and is potentially important for vaccine development.**

The identification and analysis of antigenic sites on a protein and ultimately the ability to predict their location is central to a wide range of problems in fundamental and applied immunology. The molecular basis of antigen processing and recognition is an example of the former (1–3); vaccine development is an example of the latter (4). The emergence of hybridoma technology and the consequent availability of monoclonal antibodies have greatly facilitated the search for sites recognized by antibodies, and the antigenic architecture of a number of proteins has now been mapped in considerable detail (5). Careful examination of the data thus generated indicates that antigenic sites are generally located on the protein surface (5, 6) in regions of relatively high segmental flexibility (7, 8) and hydrophilicity (9). The majority of the exposed surface may be antigenic for antibodies.

In contrast to the information and emerging concepts on antibody antigenicity, data on antigenic sites recognized by T cells are scarce, and potentially predictive concepts are essentially nonexistent. The latter deficiency is to some extent related to the former, and both are linked to the relative complexity of the T-cell response. Unlike B-cell immunoglobulin, which can recognize native, solubilized antigen, recognition of antigen by T-cell receptors requires that it be proteolytically processed or otherwise unfolded by accessory cells, such as macrophages, B cells, or dendritic cells, and that the antigenic segments thus produced be presented to T cells on the surface of an accessory cell in association with a major transplantation antigen, such as the murine Ia or the human HLA-D region antigens (1, 3, 10, 11). This complexity, coupled with the fact that the T-cell receptor has only recently become amenable to structural analysis, has also made determination of equilibrium constants for binding of free antigen to T-cell receptors difficult. Thus, quantitative studies of recognition by T-cell receptors, even at a phenomonological level, have progressed much more slowly than those for antibodies.

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Although the T-cell receptor–antigen system is in many respects more complex and less well characterized than the antigen–antibody system, it is in one important respect easier to analyze. A key observation first made a quarter of a century ago (12) and generalized to many antigens since then (reviewed in ref. 13) indicates that T cells do not distinguish between native and denatured tertiary structures of the protein, whereas antibodies usually distinguish among tertiary conformations exquisitely. This finding is probably explained by the fact that T cells see only processed antigen, which, by virtue of its relatively short length, is less likely than the native protein to have a unique highly stable tertiary structure. Thus, unlike the sites seen by antibodies, sites seen by T cells reflect lower-order structural properties of the sequence, and since these are generally easier to predict than tertiary structure, sites seen by T cells should actually be more predictable, despite the complex interactions, than those seen by antibodies.

The above observations notwithstanding, the specificity of the interaction between T-cell receptors and antigen does suggest the presence of regular order that would serve to distinguish one segment from another. Moreover, the fact that T-cell receptors do not recognize soluble antigen, but only antigen in association with a presenting cell, suggests that such order might not be present in solution but might instead be induced and stabilized by interactions with hydrophobic surface structures of the presenting cell. We would then expect one face of a regularly ordered secondary structure to consist of relatively hydrophobic residues. Polar residues on the opposite face would permit the type of bonding that could confer specificity on the interaction with the T-cell receptor. Thus, we propose that a critical requirement for a T-cell antigenic site is the ability to form a stable amphipathic structure—i.e., a structure in which the hydrophobic and hydrophilic residues tend to occur on opposite faces. An optimal sequence would be one in which the hydrophobicity of residues alternates with a frequency similar to the number of residues per turn in a regular structure—e.g., a period of 3.6 residues is consistent with an α-helix, a period of 2 residues with a β structure, and so on.

These considerations lead us to propose the following molecular concept, which, if valid, greatly simplifies the problem of predicting T-cell antigenic sites. (*i*) Relatively local secondary structural features are a major determinant of antigenic sites recognized by T cells. (*ii*) These features reflect periodicity in the hydrophobic profile of the segment. (*iii*) As a consequence of (*ii*), T-cell antigenic sites are likely to be *amphipathic structures* that are *induced and stabilized by hydrophobic interactions with* structures on the surface of *the presenting cell.*

In the remainder of this paper we test this hypothesis by determining the periodicity of hydrophobic residues in the amino acid sequences of protein antigens, identifying segments with periodicity corresponding to α or β structure, and then asking whether the known T-cell sites reported in the literature correlate with these segments of sequence. The

degree of correlation is assessed quantitatively by calculating the probability of the T-cell site falling within such a region by random chance. The results obtained by using available data on T-cell antigenic sites support the hypothesis with a level of significance that is generally considerably better than 1%.

METHODS

The most direct and rigorous way to determine whether a property of a sequence varies with a given frequency is to assign a numerical value to the property and then to compute the so-called power spectrum or correlation function of the numerical sequence [see, for example, Lin and Segel (14)]. This procedure allows one to determine whether sinusoidal variation of the property in the frequency range of interest is greater than in other frequency ranges. The standard method consists of dividing the sequence of L residues into $L - l + 1$ blocks of length l (typically 7)—the first block extending from residue 1 to residue l, the second from residue 2 to residue $l + 1$, and so on. For each block we determine the extent to which the pattern of hydrophobic residues occurs with a regular periodicity. The dominant periodicity for each block is assigned to the position of its central residue {i.e., residue $[k + (l - 1)]/2$ for block k} and runs of similar periodicity are sought.

To make these ideas precise, let h_j be the hydrophobicity of the jth residue and let $\bar{h}_k$ be the average hydrophobicity of the kth block. Then the intensity of the kth block contributed by a hydrophobic oscillation that repeats every θ degrees is (15–17)

$$I(k, \theta) = \left\{\left[\sum_{j=k}^{k+l-1} (h_j - \bar{h}_k)\sin(2\pi\theta j/360)\right]^2 + \left[\sum_{j=k}^{k+l-1} (h_j - \bar{h}_k)\cos(2\pi\theta j/360)\right]^2\right\}^{1/2}. \quad [1]$$

For each block we record the periodicity, $360/\theta$, at which the intensity is highest. For reasons that will soon become clear, we are particularly interested in those blocks for which the highest intensity is in some interval $100° - \Delta\theta \leq \theta \leq 100° + \Delta\theta$, centered on the periodicity of 3.6 residues per 360° turn of an α-helix (i.e., $\theta = 360°$ per turn ÷ 3.6 residues per turn = 100° per residue). For convenience in discussing and displaying the results, we denote by a 1 the central residues of blocks that meet this intensity condition and by 0 those that do not. Isolated 1s in a field of 0s or intermittent 1s would clearly be an unreliable condition for antigenic candidacy. Since antigenic sites are generally composed of at least 5 residues (5, 18), we seek stretches of five or more contiguous 1s. The central questions to be evaluated concern the frequency with which such runs coincide partially or completely with T-cell antigenic sites and the probability that such coincidences would have occurred at random, given the total number of high-intensity 3.6 period regions associated with the entire sequence.

For the purpose of calculating the probability, we considered the entire reported antigenic site extended on either side to the end of the overlapping block of 1s. Let w be the observed number of 1s in a region of length r, let s be the length of the observed contiguous block, and let p be the fraction of 1s in the entire sequence. Then the probability v of observing at least w 1s in r including a block of contiguous 1s at least s long is

$$v = p^r + \sum_{j=w}^{r-1} p^j(1 - p)^{r-j} \sum_{k=s}^{j} (r - j + 1)\binom{r - k - 1}{j - k}. \quad [2]$$

The required probabilities were also calculated by using Monte Carlo methods.

Finally we note that the amphipathic concept can be broadened somewhat by considering a change from polar to apolar that occurs sequentially rather than periodically (19). For this alternative type of amphipathicity we would compare the average hydrophobicity of the first half of a block of residues with the average hydrophobicity of the second half. The quantity of interest is then the *differential hydrophobicity* (the difference in average hydrophobicities between the two halves), those cases in which the difference exceeds some preassigned threshold being candidates for sites. As we indicate below, 11 antigenic sites tend to conform to periodic amphipathicity, and 1 conforms to sequential amphipathicity.

RESULTS

An analysis of sperm whale myoglobin using Eq. 1 indicates three prominent stretches with periodicity of 3.6 residues per turn (Fig. 1): from residues 59 to 81, from residues 104 to 114, and from residues 130 to 143. The latter two contain known T-cell immunodominant sites (23–26); the antigenicity of the former is not known. Close to 50% of the residues in the myoglobin sequence were scored as 1, reflecting its high α-helical content. With this percentage of 1s, the probability that each of the two stretches overlying the observed antigenic sites could have occurred at random is 3.3×10^{-4} and 0.01, respectively, so that the probability that they could both have occurred at random is $\approx 3.3 \times 10^{-6}$. A similar analysis for periodicity of 2 residues per turn (180°) showed no correlation with known antigenic sites (data not shown).

Although the numerical significance of the result for myoglobin is striking, it of course sheds little light on the generality of the concept. High-intensity amphipathicity might be reliable for a restricted class of molecules, including myoglobin, possessing appropriate structural features, but unreliable outside that class. It would be surprising if the concept applied to all molecules and equally surprising if it applied only to myoglobin.

Limited insight into the extent and domain of validity of the amphipathic hypothesis can be obtained by analyzing the six proteins possessing the 12 antigenic sites that have thus far been identified. Of the 12 sites (Table 1) 10 were predicted correctly with probabilities of random occurrence ranging for 9 of them from 0.03 to 3×10^{-5} (Table 2), all having

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          1         2         3         4
1         0         0         0         0
VLSEGEWQLVLHVWAKVEADVAGHGQDILIRLFKSHPETL
   1001001000000000000111011011001000 11

          5         6         7         8
          0         0         0         0
EKFDRFKHLKTEAEMKASEDLKKHGVTVLTALGAILKKKG
1110010000000000001111111011011111111111

                    1         1         1
          9         0         1         2
          0         0         0         0
HHEAELKPLAQSHATKHKIPIKYLEFISEAIIHVLHSRHP
1000000111010101100000011111111111000000

          1         1         1
          3         4         5
          0         0         0
GDFGADAQGAMNKALELFRKDIAAKYKELGYQG
1000000001111111110111010000011
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FIG. 1. Sperm whale myoglobin sequence (20, 21) showing residues centered on blocks of high-intensity periodicity. The 1s indicate the centers of seven-residue blocks with dominant periodicity of hydrophobicity in the range of 100 ± 20°, corresponding to that of an α-helix. Numerical values for hydrophobicity were assigned according to the scale of Kyte and Doolittle (22). Blocks tested around observed T-cell antigenic sites (Table 2) are underlined.

Table 1. Correlation of amphipathicity with T-cell antigenic sites

Molecule	Blocks of residues tested for correlation
Hen lysozyme (p = 0.31)	43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 1 1 1 1 1 1 0 0 0 1 1 1 0 0 0 0 0 0 0 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87 88 89 90 1 1 1 1 1 0 0 0 0 0 0 1 1 1 0 0 0 0 0 91 92 93 94 95 96 97 98 99 100 101 0 0 0 1 1 1 1 1 1 1 1 110 111 112 113 114 115 116 117 1 0 0 1 0 0 0 0
Ovalbumin (p = 0.52)	323 324 325 326 327 328 329 330 331 332 333 334 335 336 337 0 1 1 1 1 1 0 0 0 0 0 0 0 1 1 338 339 340 341 342 343 344 1 1 1 1 1 1 1
Pork or beef insulin (p = 0.61)	A: 4 5 6 7 8 9 10 11 12 13 14 15 16 17 1 1 1 1 1 1 0 1 1 0 1 1 1 1 B: 5 6 7 8 9 10 11 12 13 14 15 16 1 1 1 1 1 1 1 1 1 0 1 1
Human influenza hemagglutinin 1 (p = 0.58)	111 112 113 114 115 116 117 118 119 120 121 122 123 1 0 0 0 0 0 1 1 1 1 1 1 1 129 130 131 132 133 134 135 136 137 138 139 140 141 1 1 0 1 1 0 1 1 1 1 1 1 1 302 303 304 305 306 307 308 309 310 311 312 313 1 1 1 1 1 1 1 0 0 0 0 0

Blocks were chosen to include all of the published antigenic site (Table 2) with the remainder of any string of continuous 1s overlapping the site. p is the fraction of 1s in the entire protein sequence (see Eq. 2).

periodicities compatible with an α-helical repeat. Pigeon cytochrome c, which has a COOH-terminal site that has been analyzed in great detail, showed neither 100° nor 180° periodicity. The analysis did, however, locate a string of four blocks centered on residues 97–100 having dominant periodicities of 120 ± 15°, corresponding to a 3_{10}-helix. Although the probability of chance occurrence of such a run was found to be 0.02, we regard the result as ambiguous because of the difficulty in applying this method to a site that extends to the three residues at the COOH terminus where end effects make utility of the method doubtful.

For the remaining site (surrounding residues 113 and 114 of hen lysozyme) spectral analysis (Eq. 1) does not identify amphipathicity for any periodicities associated with regularly ordered structures. This site is, however, amphipathic in the extended sense, in terms of a sequential change in hydrophobicity as outlined above. We examined the differential hydrophobicity of every 8-residue block in the sequence of hen lysozyme and found that the maximum differential (−20.3 on the Kyte–Doolittle scale) occurred in the block of residues 107–114. There are only two other sites in lysozyme with differentials close to this (−19.0 and −19.1). One occurs in the known antigenic site 46–61 (19), and the other has not been studied.

What is the likelihood that a differential hydrophobicity as high as −20.3 would occur by chance in a sequence with the composition of lysozyme? The average hydrophobicity of residues 107–110 differs from the average hydrophobicity of residues 111–114 by an amount that is 2.42 standard deviation units above the mean difference expected for adjacent blocks

Table 2. Coincidence of T-cell epitopes with high-intensity periodicity of 3.6 residues per turn

Molecule	Observed antigenic sites	Refs.	Amphipathic regions	Probability of random coincidence	Joint probability*
Sperm whale myoglobin	Around 109	23, 24	104–114	3.3×10^{-5}	
	132–145	25, 26	130–145	0.010	3.3×10^{-6}
Hen lysozyme	46–61	19	43–61	0.001	
	74–96	27	72—101	3×10^{-5}	9×10^{-8}
	Around 114	28	None found†	NA	
Pigeon cytochrome c	93–103	29	None found‡	NA	
Ovalbumin	323–339	30	323–344	0.008	8×10^{-3}
Pork or beef insulin	A: 8–10	10	A: 4–17	0.040	6.8×10^{-4}
	B: 5–16	31	B: 5–16	0.017	
Human influenza hemagglutinin 1	111–119	32	111–123	0.1	3×10^{-4}
	Around 135	33	129–141	0.03	
	302–313	33, 34	302–313	0.1	
			302–308	0.02	

NA, not applicable.

*Products of the probabilities in the preceding column for the same protein. The number for lysozyme was multiplied by $\binom{3}{2} = 3$ since only 2 of the three sites were predicted.

†This third lysozyme site did, however, fit the sequential type of amphipathicity.

‡Although amphipathicity corresponding to α structure was not found, a periodicity corresponding to a 3_{10}-helix was found in the vicinity of the site.

(i.e., likelihood of <2%). This statistic was obtained by generating 20 random sequences with the same amino acid composition as lysozyme and counting the fraction of times blocks with the given hydrophobicity variation occurred. In a sequence of 127 blocks, which is the case for lysozyme, 1 or 2 are expected by chance to have variations in hydrophobicity that are greater than 2.42 standard deviations from the mean on a Gaussian curve. The probability that such a block will overlap an antigenic site 5 residues long by chance is about 3%.[‡] In our judgment these results for hen lysozyme tend to support the suggestion (19) that the abrupt change in hydrophobicity in the vicinity of the antigenic site plays a biological role somewhat similar to the one we have been describing. It is important to note that even if the standard deviation were lower and several blocks with hydrophobic changes greater than that at the known antigenic site occurred, that would not necessarily militate against the block concept, but it would predict that if the idea is valid, additional antigenic sites should be present, coinciding with those blocks of higher standard deviation.

DISCUSSION

The above analysis is in accord with a growing body of evidence that implicates amphipathicity as a major structural feature in determining the activity of small- and intermediate-sized peptides that interact with membranes (35). The hypothesis that antigenic sites are strongly amphipathic is thus consistent with numerous observations on other small membrane-associated peptide systems and suggests an important role for amphipathicity in molecular models of T-cell activation.

Our results strongly suggest the presence of a class of proteins, perhaps large, for which immunodominant antigenic sites uncovered by macrophage processing are amphipathic. In fact, previous studies of antigen processing for stimulation of T-cell clones specific for myoglobin (36) as well as studies using T cells specific for lysozyme (19) and tyrosine azobenzenearsonate (37) have led to the suggestion (3, 36) that the purpose of processing is to unfold the antigens in order to expose critical residues, especially hydrophobic residues that normally would not be directly accessible to a cell. In the case of myoglobin, the antigenic site residues 132–145 are encompassed within an amphipathic α-helical segment of the native structure (25, 26, 38), with the hydrophilic side containing the critical Lys-140 residue already exposed to solvent, while the hydrophobic side faces the interior of the protein. It is the latter hydrophobic side that is exposed by processing. Thus, processing may select for amphipathic regions, exposing hydrophobic residues and making them available for interaction with the Ia or cell membrane on the presenting cell.

A striking aspect of the results is that the periodicity of hydrophobic residues in the vicinity of antigenic sites corresponds to that of an α-helix, occurring in the range of 100 ± 20°. Several significant amphipathic runs of 180° periodicity (i.e., consistent with β structure) also occur, but in only one case—residues 87–92 of hen lysozyme—was coincidence with a known immunodominant site found (data not shown). The significance of these observations is not clear. There is some tendency toward sites being in α-helical segments in the native structure, but it is not strong enough to explain the results. For myoglobin the known T-cell sites are in α-helical regions in the native molecule (23–26, 38). The same appears to be true for cytochrome *c* (39)—although end effects in this case make calculation of amphipathicity difficult and the utility of the concept doubtful. Nevertheless, calculations by Pincus *et al.* (39) suggest a correlation between helix-forming tendency of peptide homologues of the COOH-terminal region of cytochrome *c* and their ability to stimulate T cells specific for cytochrome. Structural information is not available for ovalbumin. For insulin, most of the B-chain site—in particular, residues 9–16—is α-helical, but the A loop is not helical. For lysozyme, however, one site is not helical in the native structure, and the other is only partially helical (40). In influenza hemagglutinin, none of the sites is helical (41).[§] Nevertheless, the correlation with 100° periodicity holds.

Perhaps *what is important is not necessarily the native secondary structure* in the intact protein *but the ability of a peptide segment to form an α-helix-like structure induced and stabilized by the amphipathic environment at the surface of the antigen-presenting cell.* The periodicity of hydrophobicity we observe would favor such induction and stabilization of an α-helix. We thus conclude that what may be of fundamental importance is the ability of a peptide after processing to form a regularly ordered structure in conjunction with the presenting cell surface and that periodicity is important insofar as it facilitates meeting this requirement in the amphipathic environment of the presenting cell surface. The one exception, the A loop of insulin, which cannot be induced to form an α-helix because of the constraint of the disulfide bond, is already stabilized in an amphipathic secondary structure. We do not rule out the possibility that sequences with 180° periodicity might also be antigenic. Indeed, the possibility that they are is appealing, since they can also be hydrophobically stabilized as regularly ordered structures. The relative frequency in our limited sample suggests, however, that they would tend not to be immunodominant. These correlations might indicate something fundamental about the chemistry of antigen presentation that warrants experimental exploration.

We wish to be clear about the constraints on the interpretation of the amphipathic concept in its current form. First, amphipathicity is not the only factor that determines antigenicity for T cells. Self-tolerance, *Ir* genes, and other factors in the host clearly play a major role. Thus, for example, the three antigenic sites of lysozyme are each immunodominant in a different mouse haplotype: either $H\text{-}2^k$ (19), $H\text{-}2^b$ (27), or $H\text{-}2^d$ (28). Similarly, although both immunodominant sites of myoglobin are recognized by $H\text{-}2^d$ mice, the Glu-109 site is immunodominant in association with $I\text{-}A^d$ and the 132–146 site with $I\text{-}E^d$ (25). Thus, recombinant mice of the B10.GD strain ($I\text{-}A^d$, *I-E* not expressed) respond to the former but not the latter (24, 25). Recognition of *all* amphipathic sites by a *single* animal or inbred strain should, therefore, not be expected.

A second important constraint is related to current limitations in data and involves the distinction between the test of a concept on a given data set and its use as a predictive tool. For example, approaches that have been suggested for locating antigenic sites bound by antibodies (7–9) all demonstrate that known antigenic sites fall into regions characterized by a certain property, such as hydrophilicity or mobility. This is not the same as demonstrating that all regions with the given property are antigenic sites. Our work so far is subject to the same limitation. The results indicate that with high probability, when an antigenic site occurs, it is associated with a contiguous stretch of blocks of amphipathic residues—i.e., if a run of residues is antigenic, it will be very likely amphipathic. The analysis does *not necessarily* indicate the converse—that if a run is amphipathic, it will be antigenic.

[‡]A block of residues r long can be placed in $L - r + 1$ ways along a protein molecule of L residues. Of these, $r + n + 1 - 2l$ overlap an antigenic site of length n by at least l residues. An overlap probability of 0.03 is obtained with $r = 8$ and $l = n = 5$.

[§]T-cell sites were described for the H1 subtype (32, 33) or H3 subtype (34) of hemagglutinin, whereas the crystal structure was that of the H3 subtype only (41).

The reason of course is that all maximally amphipathic sites have not been tested for antigenicity. Thus, we cannot claim, for example, that the other blocks of 1s in myoglobin contain all or part of an antigenic site, but we do predict that if there is another immunodominant site, it is likely to be in one of those regions. Extensions that would permit assessment of the reliability of converse conclusions are clearly of central interest to a number of problems, including vaccine development. This will require testing many sites and, as noted above, many strains of animals. Even now, however, the concept suggests testable predictions about the molecular role of antigen processing, and it considerably simplifies the search for immunodominant sites. If most T-cell antigenic sites are amphiphathic, as our results suggest, then the fraction of the sequence of any given protein that one must examine to find most of its antigenic sites is markedly reduced.

Note Added in Proof. The following points should be noted. (*i*) Since a 1 denotes the center of a block of 7 residues, the periodicity extends 3 residues beyond the last 1 at each end of a string of 1s. Thus, the recent report of Allen *et al.* (42) is compatible with Table 1. (*ii*) After this paper was submitted, we became aware of a further analysis by Watts *et al.* (43) of the ovalbumin site examined here. Their interpretation and ours support one another.

We have recently become aware that Drs. Mathew Pincus, Ronald Schwartz, and John Weinstein have also been considering the role of amphipathicity in antigen presentation. We are deeply indebted to Dr. Schwartz for reading and discussing the manuscript, for providing information on cytochrome-related antigenic sites, and for suggesting the possibility that such sites might display periodicity characteristic of a 3_{10}-helix. We also thank the following National Institutes of Health colleagues for reading and commenting on the manuscript: Drs. Jeffrey Bluestone, Kemp Cease, David Davies, Jacob Maizel, Edward Max, and Alan Schechter.

1. Unanue, E. R. (1984) *Annu. Rev. Immunol.* **2,** 395–428.
2. Ziegler, K. & Unanue, E. R. (1981) *J. Immunol.* **127,** 1869–1875.
3. Berzofsky, J. A. (1985) in *The Year in Immunology 1984-85*, eds. Cruse, J. M. & Lewis, R. E., Jr. (Karger, Basel, Switzerland), pp. 18–24.
4. Lerner, R. A. (1984) *Adv. Immunol.* **36,** 1–44.
5. Benjamin, D. C., Berzofsky, J. A., East, I. J., Gurd, F. R. N., Hannum, C., Leach, S. J., Margoliash, E., Michael, J. G., Miller, A., Prager, E. M., Reichlin, M., Sercarz, E. E., Smith-Gill, S. J., Todd, P. E. & Wilson, A. C. (1984) *Annu. Rev. Immunol.* **2,** 67–101.
6. Berzofsky, J. A. (1985) *Science*, **229,** 932–940.
7. Tainer, J. A., Getzoff, E. D., Alexander, H., Houghten, R. A., Olson, A. J., Lerner, R. A. & Hendrickson, W. A. (1984) *Nature (London)* **312,** 127–133.
8. Westhof, E., Altschuh, D., Moras, D., Bloomer, A. C., Mondragon, A., Klug, A. & Van Regenmortel, M. H. V. (1984) *Nature (London)* **311,** 123–126.
9. Hopp, T. P. & Woods, K. R. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 3824–3828.
10. Rosenthal, A. S. (1978) *Immunol. Rev.* **40,** 136–156.
11. Benacerraf, B. (1978) *J. Immunol.* **120,** 1809–1812.
12. Gell, P. G. H. & Benacerraf, B. (1959) *Immunology* **2,** 64–70.
13. Berzofsky, J. A. (1980) in *Biological Regulation and Development*, Goldberger, R. F. (Plenum, New York), Vol. 2, pp. 467–594.
14. Lin, C. C. & Segel, L. A. (1974) *Mathematics Applied to Deterministic Problems in the Natural Sciences* (MacMillan, New York), pp. 171–181.
15. Eisenberg, D., Weiss, R. M. & Terwilliger, T. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 140–144.
16. Finer-Moore, J. & Stroud, R. M. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 155–159.
17. Klein, P., Kanehisa, M. & DeLisi, C. (1984) *Biochim. Biophys. Acta* **787,** 221–226.
18. Berzofsky, J. A. & Berkower, I. J. (1984) in *Fundamental Immunology*, ed. Paul, W. E. (Raven, New York), pp. 595–644.
19. Allen, P. M., Strydom, D. J. & Unanue, E. R. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 2489–2493.
20. Edmundson, A. B. (1965) *Nature (London)* **205,** 883–887.
21. Herrera, A. E. R. & Lehmann, H. (1974) *Biochim. Biophys. Acta* **336,** 318–323.
22. Kyte, J. & Doolittle, R. F. (1982) *J. Mol. Biol.* **157,** 105–132.
23. Berkower, I., Buckenmeyer, G. K., Gurd, F. R. N. & Berzofsky, J. A. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 4723–4727.
24. Berkower, I., Matis, L. A., Buckenmeyer, G. K., Gurd, F. R. N., Longo, D. L. & Berzofsky, J. A. (1984) *J. Immunol.* **132,** 1370–1378.
25. Berkower, I., Kawamura, H., Matis, L. A. & Berzofsky, J. A. (1985) *J. Immunol.* **135,** 2628–2634.
26. Berkower, I., Buckenmeyer, G. & Berzofsky, J. (1985) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **44,** 1312.
27. Manca, F., Clarke, J. A., Miller, A., Sercarz, E. E. & Shastri, N. (1984) *J. Immunol.* **133,** 2075–2078.
28. Katz, M. E., Maizels, R. M., Wicker, L., Miller, A. & Sercarz, E. E. (1982) *Eur. J. Immunol.* **12,** 535–540.
29. Hansburg, D., Fairwell, T., Schwartz, R. H. & Appella, E. (1983) *J. Immunol.* **131,** 319–324.
30. Shimonkevitz, R., Colon, S., Kappler, J. W., Marrack, P. & Grey, H. (1984) *J. Immunol.* **133,** 2067–2074.
31. Thomas, J. W., Danho, W., Bullesbach, E., Föhles, J. & Rosenthal, A. S. (1981) *J. Immunol.* **126,** 1095–1100.
32. Hackett, C. J., Dietzschold, B., Gerhard, W., Ghrist, B., Knorr, R., Gillessen, D. & Melchers, F. (1983) *J. Exp. Med.* **158,** 294–302.
33. Hurwitz, J. L., Heber-Katz, E., Hackett, C. J. & Gerhard, W. (1984) *J. Immunol.* **133,** 3371–3377.
34. Lamb, J. R., Eckels, D. D., Lake, P., Woody, J. N. & Green, N. (1982) *Nature (London)* **300,** 66–69.
35. Kaiser, E. T. & Kézdy, F. J. (1984) *Science* **223,** 249–255.
36. Streicher, H. Z., Berkower, I. J., Busch, M., Gurd, F. R. N. & Berzofsky, J. A. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 6831–6835.
37. Godfrey, W. L., Lewis, G. K. & Goodman, J. W. (1984) *Mol. Immunol.* **21,** 969–978.
38. Takano, T. (1977) *J. Mol. Biol.* **110,** 537–568.
39. Pincus, M. R., Gerewitz, F., Schwartz, R. H. & Scheraga, H. A. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 3297–3300.
40. Phillips, D. C. (1967) *Proc. Natl. Acad. Sci. USA* **57,** 484–495.
41. Wilson, I. A., Skehel, J. J. & Wiley, D. C. (1981) *Nature (London)* **289,** 366–373.
42. Allen, P. M., Matsueda, G. R., Haber, E. & Unanue, E. R. (1985) *J. Immunol.* **135,** 368–373.
43. Watts, T. H., Gariépy, J., Schoolnick, G. K. & McConnell, H. M. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 5480–5484.