OVERLAPPING T CELL ANTIGENIC SITES ON A SYNTHETIC PEPTIDE FRAGMENT FROM HERPES SIMPLEX VIRUS GLYCOPROTEIN D, THE DEGENERATE MHC RESTRICTION ELICITED, AND FUNCTIONAL EVIDENCE FOR ANTIGEN–Ia INTERACTION

By ELLEN HEBER-KATZ, SHARON VALENTINE,
BERNHARD DIETZSCHOLD, AND CATHERINE BURNS-PURZYCKI

From The Wistar Institute of Anatomy and Biology, Philadelphia, Pennsylvania 19104

The glycoprotein D (gD)[1] of HSV is found both on the envelope of the virion and on the surface of HSV-infected cells. The binding of neutralizing antibody specific for gD results in the elimination of this virus in experimental infections (1–6). Therefore, passive transfer of neutralizing mAb will protect mice from a lethal challenge of HSV-1 and HSV-2 (7). A linear antigenic site at the NH_2 terminus of the gD molecule has been identified using anti-gD antibodies, and a synthetic peptide 23 amino acids long has been shown to mimic this site in its antibody-binding activity. Using overlapping synthetic peptides, the 23–amino acid peptide fragment has been subdivided into two major antigenic determinants for B cells, localized in one fragment encompassing residues 1–16 and in another fragment encompassing residues 8–23 (8, 9).

This 23–amino acid dominant B cell determinant has also been shown to be antigenic for class II–restricted proliferating T cells (10). This is an unusual finding since T cell and B cell antigenic sites generally do not overlap (11–16). We were therefore interested in examining the fine specificity of these T cells, their MHC restriction, and their response patterns to the series of antigenic synthetic peptides previously used in the B cell studies to eventually compare these two antigen-specific repertoires.

In the present study we found that B10.A mice immunized with the 1–23 peptide also produced T cells specific for two determinants. A T cell response to one of the determinants, 1–16, of the 23–amino acid HSV peptide was found to be MHC restricted to the I-A molecule. The response to the second of these determinants, 8–23, was exclusively restricted to the I-E molecule. Using this 1–23 HSV peptide antigen, we have found that T cell responses appear to have the same fine specificity as B cell responses, even though T cells must also

---

This work was supported by grants from R. J. Reynolds, the American Cancer Society (IM-417), and the National Institutes of Health (AI-22528 and NS-11036). Address correspondence to Dr. Ellen Heber-Katz, The Wistar Institute, 36th Street at Spruce, Philadelphia, PA 19104.
[1] *Abbreviation used in this paper:* gD, glycoprotein D.

J. EXP. MED. © The Rockefeller University Press · 0022-1007/88/02/0275/13 $2.00   275
Volume 167   February 1988   275/287

```
                          1                    7                    14                        21    23

1-23[1]* (7 Ala, 21 Asp)  Lys Tyr Ala Leu Ala Asp Ala Ser Leu Lys Met Ala Asp Pro Asn Arg Phe Arg Gly Lys Asp Leu Pro
1-23[2]‡ (7 Pro, 21 Asn)  1 - - - - - - - - - - Pro - - - - - - - - - - - - - - - - - - - - - - - - - - Asn - - 23
1-23[H]§ (7 Pro, 21 Asp)  1 - - - - - - - - - - Pro - - - - - - - - - - - - - - - - - - - - - - - - - - Asp - - 23
1-16[1] (7 Ala)           1 - - - - - - - - - - Ala - - - - - - - - - - - - - - - 16
1-16[2] (7 Pro)           1 - - - - - - - - - - Pro - - - - - - - - - - - - - - - 16
8-23[1] (21 Asp)                                    8 - - - - - - - - - - - - - - - - - - - - - - - - Asp - - 23
8-23[2] (21 Asn)                                    8 - - - - - - - - - - - - - - - - - - - - - - - - Asn - - 23
17-23[1] (21 Asp)                                                               17 - - - - - Asp - - 23
17-23[2] (21 Asn)                                                               17 - - - - - Asn - - 23
```

FIGURE 1.   Primary amino acid sequences of HSV peptides. (*) The HSV-1 sequence of gD with Ala at residue 7 and Asp at residue 21. (‡) The HSV-2 sequence of gD with Pro at residue 7 and Asn at residue 21. (§) A hybrid gD sequence from HSV-1 and HSV-2 with Pro at residue 7 and Asp at residue 21.

recognize the Ia molecule. Furthermore, the I-E-restricted response is MHC degenerate in that all T cell clones specific for 8–23 responded to antigen on APCs of different MHC haplotypes and these clones showed different antigenic fine specificities on the different APCs. These results are surprisingly reminiscent of the findings with the COOH-terminal cytochrome c–specific T cell response (17) and are consistent with the original interpretation of the interaction of antigen and Ia at the time of T cell activation (18), which has been recently supported by physical interaction data (19–24).

However, studies involving point mutations in the Ia molecules and the use of synthetic variants of peptide antigens have led to a narrower interpretation that the specific amino acid substitution that led to functional vs. nonfunctional complexes also maps the critical contact sites (25–27). That is, that these amino acids are within the agretope and desetope as such. We present evidence that at least for the agretope, this interpretation is not sustainable.

Materials and Methods

Animals.   B10/SgSn (bbbb), B10.A/SgSn (kkkd), and B10.A(5R)/SgSn (bbkd) mice were obtained from the The Jackson Laboratory, Bar Harbor, ME. B10.S(9R) (sskd) mice were obtained from R. Schwartz at the National Institutes of Health and B10.A(4R) (kkbb) mice were obtained from K. Blank, University of Pennsylvania, Philadelphia, PA. BALB/c (dddd) were obtained from Charles River Breeding Laboratories, Inc., Wilmington, MA. Letters indicate the haplotype source of the K, I-A, I-E, and D subregion alleles of the murine MHC.

Antigens.   Peptides were synthesized as previously described (10) and are shown in Fig. 1. Synthesis was performed using the Merrifield solid-phase method (28). The completed peptides were simultaneously deprotected and cleaved from the resin using anhydrous hydrogen fluoride–containing thioanisole. The crude peptides were purified by gel filtration on Bio-Gel P-2 or P-4 (Bio-Rad Laboratories, Richmond, CA) columns. Homogeneity of the peptides was demonstrated by thin-layer chromatography, amino acid analysis, and amino acid sequencing. The purified peptides were analyzed by thin-layer gel chromatography using two solvent systems (l-butanol/acetic acid/water = 4:1:1 or ethylacetate/pyridine/acetic acid/water = 60:20:6:11), silica gel 60 precoated aluminium sheets (Merck & Co., Inc., Rahway, NJ) and ninhydrin or chlorine-tolidine staining. Amino acid composition was determined after acid hydrolysis in 6 N HCl at 100°C in evacuated sealed tubes for 24 h. Amino acid analyses were carried out using a gas-phase sequencer (model 470A; Applied Biosystems, Inc., Foster City, CA). After lyophilization under

TABLE I

MHC Restriction and Antigen Specificity of B10.A T Cell Hybridomas

Clone no.: Antigen:	2.3.7			2.3.8			2.3.20		
	1-23 (H)	1-16 (2)	8-23 (1)	1-23 (H)	1-16 (2)	8-23 (1)	1-23 (H)	1-16 (2)	8-23 (1)
APC									
B10.A (kkkd)	93.4*	2.5	85.8	31.9	1.3	41.3	10.5	0.9	3.7
B10.A(4R) (kkbb)	1.2			1.1			0.6		
B10.A(5R) (bbkd)	48.9			13.5			4.0		
B10.A + 17.3.3	3.8			0.8			0.8		
B10.A + 10.2.16	71.2			25.2			7.5		
BALB/c (dddd)	0.9			1.2			1.1		

$*$ cpm $\times$ 10^{-3}

standard conditions, the peptide content of the samples was determined by amino acid analysis.

Lymph Node T Cell Proliferative Responses. Mice were immunized in the hind footpads with 100 μg of 1-23(H), a hybrid of the HSV-1 and HSV-2 gD sequences (9, 10), emulsified in CFA. 12-14 d later, popliteal and inguinal lymph nodes were removed and T cells prepared by passing the cell suspensions over a nylon-wool column (29). T cells (4 $\times$ 10^5/well) were then cultured in 96-well flat-bottomed plates (Nunc, Roskilde, Denmark) in the presence of 2 $\times$ 10^5 X-irradiated normal spleen cells with or without antigen. After 3 d of culture, proliferation was determined as [^{3}H]thymidine incorporation into DNA.

Preparation of T Cell Hybridomas (17). Nylon wool–purified T cells (5 $\times$ 10^6) from peptide-immunized animals were cultured with X-irradiated syngeneic spleen cells (3,000 rad, 2 $\times$ 10^6) plus antigen for 3 d in 24-well plates, harvested, and mixed with the HAT-sensitive BW5147 AKR thymoma line. Fusion was carried out with polyethylene glycol 1,000 (J. T. Baker Chemical Co., Phillipsburg, NJ), 30% (vol/vol) for 8 min. The fused cells were then plated at a density of 5 $\times$ 10^5/well of a 96-well plate (Costar, Cambridge, MA). After 6 d, hydridomas were picked and expanded in 24-well plates (Costar) and then tested for antigen-specific growth factor production. Positive hybridomas were then cloned by limiting dilution at 1.0, 0.5, and 0.25 cells/well.

IL-2 production was assayed by first setting up a primary culture in which 5 $\times$ 10^4 T cell hybridomas were mixed with 2 $\times$ 10^5 X-irradiated syngeneic spleen cells with or without antigen at a given concentration. After 2 d, the supernatant from the first culture plate was transferred to a second plate and 4 $\times$ 10^3 CTLL cells (30) were added. After 2 d, the degree of CTLL stimulation was measured by [^{3}H]thymidine incorporation into DNA.

Antibody Blocking Experiments. mAb 17.3.3. (anti-I-E; reference 31) and 10.2.16 (anti–I-A^{k,f,j,r,s,u}; reference 32) were used. Various concentrations of ascitic fluid were added at the initiation of T cell culture and blocking was determined by the percent of the response obtained in the presence of antigen plus anti-Ia divided by the response with antigen alone.

Results

B10.A T Cell Hybridomas Are 8-23 Specific and I-E Restricted. The clonal B10.A T cell response to the NH$_2$ terminus of HSV gD viral glycoprotein was examined for its MHC restriction and antigen specificity, and the assortment relationships of the two by generating T cell hybridomas from mice immunized with the 1-23(H) peptide. The hybridomas were selected for responsiveness to 1-23(H) in the presence of B10.A APC. Analysis of the antigen specificity and the MHC restriction of several of these clones (Table I) revealed that (*a*) they

TABLE II

Degree of MHC Degeneracy Found in B10.A T Cell Hybrids

Exp.	Positive hybrids on B10.A APC kkkd	Positive hybrids on B10.A(5R) APC bbkd	Positive hybrids on B10.A(4R) APC kkbb	Minimum degeneracy
	n	n	n	%
1	13	10	0	77
2	11	11	0	100
3	10	6	0	60
4	37	20	0	54
5	17	10	0	59

T cell hybridomas (5 × 10⁴ per well) were tested with 50 μg/ml of the 1–23(H) peptide with B10.A, B10.A(5R), and B10.A(4R) APCs in the form of 2 × 10⁵ X-irradiated spleen cells. After 2 d, supernatants were tested for IL-2 by a CTLL assay.

could respond to the COOH-terminal 8–23 peptides, but not the NH_2-terminal 1–16 peptides, and (b) they were I-E restricted. Thus, the clones could not respond to antigen on B10.A(4R) APCs, which share only the I-A^K with B10.A (they do not express the I-E molecule), and their antigen-specific response on B10.A APC could be blocked by 17.3.3, an anti-I-E^k mAb, but not 10.2.16, an anti-I-A^k mAb. Furthermore, these clones responded to antigen on B10.A(5R), which share I-E determinants with B10.A. One explanation of this last result is that it represents an MHC-degenerate response in that T cells respond to antigen on cross-reactive I-E molecules. However, another explanation is that these T cells recognize antigen in association with the Eα molecule, which is invariant among all I-E (+) mouse strains. We therefore tested the response of these hybrids to antigen on an I-E (+) APC population derived from BALB/c (Table I). The clones did not respond to antigen in association with the I-E^D molecule, and can be explained by MHC degeneracy, and not by responsiveness to Eα.

The extent of MHC degeneracy is seen in Table II, where five separate T cell fusions between BW5147 and B10.A T cells specific for 1–23(H) revealed 54–100% of the clones reactive to antigen in the presence of B10.A(5R) (bbkd) APCs. All of the clones were nonreactive to antigen on B10.A(4R) APCs, supporting the idea that they are I-E restricted. Also, among those clones that were negative on B10.A(5R) APCs, there were clones reactive to antigen on B10.S(9R) APCs (sskd) (data not shown).

Evidence of Antigen–Ia Interaction.   To determine whether APCs of different haplotypes could affect the expressed antigen specificity of the hybrids, we tested the 8–23-specific T cells for their fine specificity using peptides of different lengths and APCs from B10.A and B10.A(5R) mice. B10.S(9R) APCs were also tested, but only for their ability to present the 1–23(H) peptide. Fig. 2 shows five different B10.A 1–23(H) clones displayed with their fine specificity patterns using two different antigen concentrations (50 and 5 μg/ml). Clones 1 and 2 are A and 5R degenerate, clone 3 is reactive to antigen on B10.A APC and both allo-reactive and antigen-reactive on B10.A(5R) APCs, clone 4 is A/9R degenerate, and clone 5 is A/5R/9R degenerate. Comparison of the B10.A- and B10.A(5R)-presented response revealed clear changes in specificity with two of
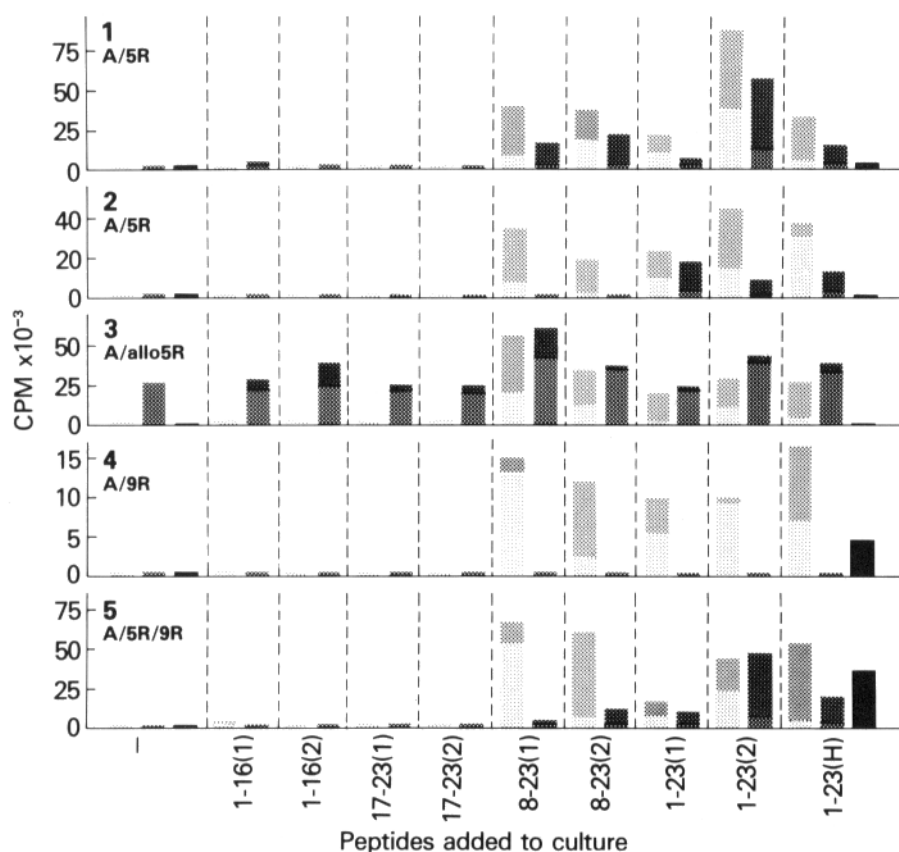
FIGURE 2. T cell hybridomas derived from B10.A mice immunized with the 1–23(H) peptide. The response of five clones to various synthetic peptides is shown in the presence of APCs from three different mouse haplotypes. For each antigen, the lightly shaded bar on the left represents the B10.A APC, the darker shaded bar on the right represents the B10.A(5R) APC, and the black bar found only with no antigen or the 1–23(H) peptide represents B10.S(9R) APC. Two different antigen concentrations are shown by the differential shading of each bar; thus, the darker shading of a bar represents 50 μg/ml and the lighter shading of a bar represents 5 μg/ml. These data represent a secondary IL-2 assay with thymidine incorporation into proliferating IL-2 indicator cells, CTLL, and derives from an initial assay with T cell hybridomas, x-irradiated spleen, and two concentrations of antigen.

the clones, 2 and 5. For both of these clones the full-length peptides and the shorter-length peptides are presented as potent antigens by the B10.A APC. The B10.A(5R) APCs, however, showed obvious differences in their presentation of these antigens. For clone 2, no response was detectable to the shorter peptides, while for clone 5 a small but reproducible response was generated by 8–23(1) and 8–23(2) on B10.A(5R) APCs. Both clones responded well to the full-length peptides on these APCs. Considering that there appears to be one receptor that recognizes both antigen and Ia, this MHC-directed antigenic fine specificity supports in a functional way the notion that antigen and Ia do interact to effect this change in specificity. The clone 5 response demonstrates the existence of an agretope in the 8–23(2) peptide (and, of course, the presence of an epitope) as presented on B10.A(5R). But, the fourfold amplification seen when the 1–23(2)
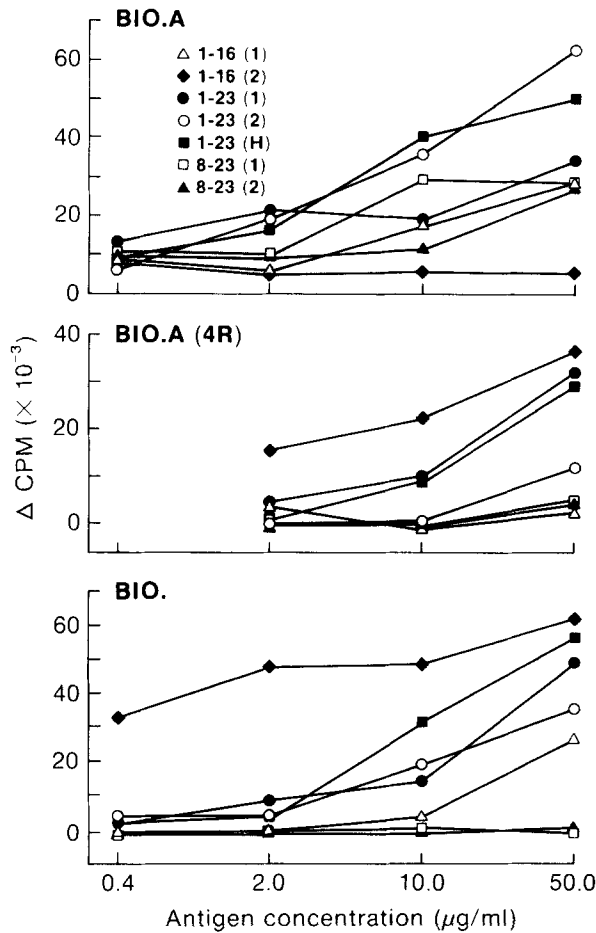
FIGURE 3. The proliferative response of T cells from B10.A (*top*), B10.A(4R) (*middle*), and B10 (*bottom*) mice immunized with the 1–23(H) peptide. T cells were cultured with X-irradiated spleen cells to which the indicated synthetic peptides were added at various concentrations to the culture.

peptide is presented on the same APC to the same T cell clone suggests strongly that the distal 1–7 contiguous sequence exerts a conformational effect. That this is not an artifact due to a difference in the physical–chemical properties of these peptides (for instance membrane solubility) is suggested by the fact that in many cases in Fig. 2, 8–23(1) and 8–23(2) induce an equal or higher response than 1–23(2).

The case for clone 2 is ambiguous since no response was seen to the shorter peptides. It can broadly be argued that either no agretope exists within 8–23(1) and 8–23(2) for B10.A(5R) and that the 1–7 sequence supplies a functional agretope, or that the presence of 1–7 conformationally alters the 8–23 region resulting in a functional agretope (an extreme version of the clone 5 case). But when the agretope is clearly located within the 8–23 sequence [B10.A(5R) APC with clone 5] the results, nonetheless, seem to indicate that nonlocal structure can have a profound effect on responsiveness.

Responsiveness to the 1–16 Peptide and I-A Restriction. We also examined the proliferative response of the whole lymph node T cell population of B10.A (kkkd) mice immunized with 1–23(H) (Fig. 3). This population responded to
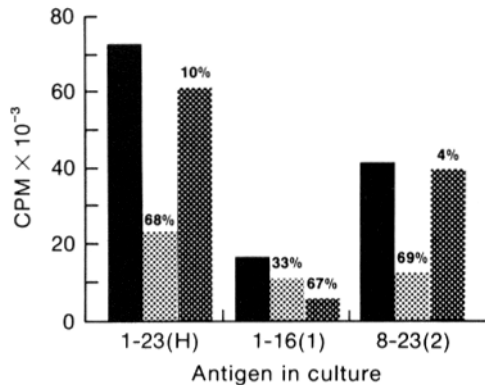
80

60

CPM × 10⁻³

40

20

0

10%

68%

33%
67%

69%

4%

1-23(H)   1-16(1)   8-23(2)

Antigen in culture

FIGURE 4.   The blocking of a T cell proliferative response from B10.A immunized with the peptide 1–23(H) using anti-Ia antibodies. The response to 50 μg/ml of 1–23(H), 1–16(1), and 8–23(2) is indicated (■). Anti–I-E mAb, 17.3.3, was added at the beginning of culture along with antigen at a final concentration of 1:1,000 (□) as was the anti-I-A mAb, 10.2.16, also at a concentration of 1:1,000 (▨). The numbers above the bars indicate the percent inhibition of the response obtained in the absence of antibody.

synthetic peptides derived from both ends of the 23–amino acid peptide; i.e., the NH_2-terminal 1–16(1) peptide and the COOH-terminal 8–23(1) and (2) peptides. Since the clonal T cell response was directed to the 8–23 peptides only, it was curious that there was a response here to the 1–16 peptide. We were interested in the MHC restriction of this response and also wanted to confirm that the 8–23 response of the hybridoma T cell clones represented the population as a whole. Results in other T cell antigenic systems (15, 43) suggested that separate determinants could be dissected by their differential Ia presentation requirements. We therefore used anti-Ia antisera (Fig. 4) to determine the contribution of the different Ia molecules to the T cell response to the 1–16 and 8–23 peptides. We found that the anti-I-E mAb 17.3.3 could block the response to 8–23 peptide, whereas the anti-I-A^k mAb 10.2.16 could not. On the other hand, the response to 1–16 was blocked more by 10.2.16 than by 17.3.3. The response to peptide 1–23(H) was blocked to the largest extent by 17.3.3. and less by 10.2.16. As a first approximation, it appears from these data that T cell recognition serves to subdivide the 1–23 molecule into two parts, each associated with a particular Ia molecule, the 1–16 determinant being recognized in association with the I-A molecule and the 8–23 determinant being recognized in association with the I-E molecule.

We attempted to derive further support for this association of an antigenic determinant and a given Ia isotype by immunizing mice that expressed only the I-A molecule with the 1–23(H) peptide. T cells of B10.A(4R) (kkbb) mice (which share the I-A^k with B10.A) and T cells of B10 (bbbb) animals, which have an I-A^b haplotype, were tested for proliferation to the panel of gD peptides (see Fig. 3). T cells from both mouse strains responded to the 1–23(H) peptide but not to the 8–23 peptides, supporting the anti-Ia blocking data. However, examination of the 1–16 responses did not lead to a clear assignment. Thus, 1–16(1) stimulated a B10.A lymph node T cell response but did not stimulate a B10.A(4R) response, which could mean that the 1–16(1) response maps to I-E^k. However, the fact that B10.A(4R) responds to 1–16(2) can be interpreted to mean that the 1–16 response does indeed map to I-A (in this case, specifically to I-A^k). However, differences in T cell repertoire, regulation, or antigen presentation, in the absence or presence of the I-E molecule, may result in this observed 1–16 fine
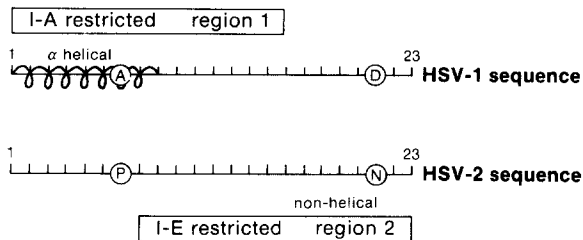
FIGURE 5. A schematic representation of the T cell determinants present on the 23-amino acid peptide derived from the NH₂ terminus of the gD from HSV-1 and HSV-2. One determinant is found in the first 16 amino acids, 1–16, and is I-A restricted. The second determinant is found in the last 16 amino acids, 8–23, and is I-E restricted. The I-A-restricted determinant appears to be helical, whereas the I-E-restricted determinant is nonhelical (10).

specificity variance. The responses observed with B10 are consistent with the 1–16 response mapping to I-A.

Taken together, the experiments above, with the caveats noted, provide evidence for association between 1–16-specific T cell responses and I-A, and between 8–23-specific T cell responses and I-E (Fig. 5).

Discussion

In the present study, we examined the T cell response to synthetic peptides corresponding to the NH₂-terminal 23 amino acids of the gD molecule of HSV-1 and HSV-2. This 23-amino-acid peptide has been shown to be composed of two major antigenic determinants, coincident for both B and T cells. In the case of B cells, mAbs made against HSV-1 and HSV-2 exist that have the same antigenic fine specificity as the B10.A T cell hybridoma clones in that they bind to the 8–23 peptides but not to the 12–23 or 1–16 peptides (reference 9, and Watari, E., unpublished data). The B cell response to the 1–16 peptide is more difficult to induce and can only be demonstrated when animals have been immunized with gD of HSV-2.

In the case of T cells, which recognize the same two determinants but in the context of MHC, each of the two antigenic determinants functionally associates with a different Ia isotype. Though a proliferative T cell response to both the 1–16 and 8–23 determinants is seen in the B10.A 1–23(H)-primed lymph node, the response to the 8–23 determinant appears to dominate as indicated by a selection for only 8–23-specific T cells in the hybridomas generated. All of these clones and the whole population of 8–23–specific T cells are I-E restricted, as they are blocked by anti-I-E antibody.

The T cell response to the 1–16 peptide associates with the I-A molecule, based both on antibody blocking data and on the finding that B10 and B10.A(4R) mice, which express only the I-A molecule, respond only to the 1–16 peptide when immunized with 1–23(H). Despite the proliferative response to this peptide in a 1–23(H) peptide–primed B10.A lymph node T cell population, we were unable to isolate any T cell hybrids with this specificity. It is possible that the 1–16 peptide response is labile since the restimulation of an in vitro 1–23(H) peptide–specific T cell culture is almost devoid of a 1–16 response (see reference 10). However, consistent with the notion that the 1–16 peptide is associated with the I-A molecule, all B10.A(5R) T cell hybridomas that are 1–16 specific are also I-A restricted (data not shown).

Fig. 5 represents a map of the antigenic determinants, their secondary structure, and their association with Ia. Structure analysis of the peptides has been determined both by Chou-Fasman predictive models (33) and confirmed by circular dichroism measurements in trifluoroethanol (10). As shown previously, we found that the 1–16 determinant is helical and immunization with a helical peptide, in particular the 1–23(1) peptide, results in a response to the helical part of the molecule. On the other hand, immunization with a nonhelical 1–23(H) peptide results in a response directed mainly to a nonhelical part of the molecule (8–23). It should be noted that in Fig. 2, the clonal 8–23-specific T cell responses to the 1–23(1) peptide are consistently low and we previously attributed this to its high helical component which affects the 8–23-specific response. The significance of a predominant secondary structure and its association with a given Ia molecule is unclear at present.

The ability of animals to recognize an antigen in association with a private determinant on the I-E molecule is not an unusual finding; it is generally accepted that T cells recognize antigen in association with private determinants of MHC-encoded molecules, a phenomenon known as MHC restriction (34–37). Rather, it is the degree of MHC degeneracy seen here that is of interest, a finding most easily understood as structural similarities of class II antigenic determinants (38, 39). Studies by Janeway et al. (40) suggested that self-MHC restriction was so precise that no degeneracy could be found in BALB/c anti-OVA clones, whereas nonself-MHC degeneracy was not uncommon in alloresponses. On the other hand, there are examples to the contrary (41–44), including the B10.A T cell response to the COOH-terminal 81–104 peptide of the cytochrome c molecule (17, 45, 46). In this case, MHC degeneracy is the rule since the response in the B10.A occurs in the presence of either B10.A and B10.A(5R) APCs, or B10.A and B10.S(9R) and/or B10.SM APCs. The same degree of MHC degeneracy is seen in the T cell response to the herpes gD NH_2-terminal 1–23 peptides with what appears to be the same MHC specificity.

MHC–antigen associations such as those observed with both of the 1–23 determinants could be due to the selection of the T cell repertoire through thymic education (47), suppression (48), T cell receptor gene usage (49), or the selective physical association of these peptides with a particular Ia molecule (18–24), also known as determinant selection (50, 51). Our data support the last possibility. We found that the antigen specificity pattern of an 8–23 peptide-specific T cell changed with the MHC haplotype of the APC, and like cytochrome c, this is another example of a change in specificity with different APCs. In this case, however, we have used a viral antigen to demonstrate this. We interpret both as a manifestation of a trimolecular complex of antigen, Ia, and T cell receptor at the time of T cell priming. One feature of this change in specificity is that with one of the clones (clone 5), B10.A APC presents both 1–23 and 8–23 peptides well, whereas the B10.A(5R) APC presents the longer 1–23 peptides far better than the 8–23 peptides.

From the findings with cytochrome c, we identified two sites on the peptide antigen: T cell binding (epitope) and Ia binding (agretope). In defining sites for the peptides in the present study, we had to deal with another difference between the herpes and the cytochrome c response. Both the antibody and the T cell

antiherpes response are sensitive to nonlocal structural changes in the peptides, making analysis of these molecules in a linear, noninteractive fashion difficult (26, 52). A linear model would predict that a mAb specific for 8–23 but not for 12–23 would almost certainly react with the 1–16 peptide, which has an 8–16 residue overlap region. This is not the case. Furthermore, the same effect is observed at the T cell level where the B10.A response is modified by amino acids outside of the determinant and has been related to conformational changes in the peptides as determined by circular dichroism measurements (10).

In this paper, we have shown that it is at least the agretope that is sensitive to conformation changes. Thus, clone 5, which recognizes 8–23(2) on B10.A(5R) APCs, gives a fourfold enhanced response to 1–23(2) on these same APCs. We must conclude that the most reasonable interpretation of this finding is that the agretope and epitope are both located in the 8–23 region, but that the agretope can be modified by the distal 1–7 sequence. The interpretation derived from other studies using either point mutations at the Ia locus or synthetic variants of peptides is that specific amino acid substitutions that lead to functional vs. nonfunctional complexes map the critical contact sites and that these amino acids are within the agretope and desetope as such (25–27). The conclusion cited above argues against such a direct interpretation. Thus, changes made in given residues resulting in a change in specificity of the T cell response may not be mapping actual intermolecular contact points in this case.

Summary

Analysis of the B10.A T cell response to synthetic peptides representing the NH_2-terminal 23 amino acids from the HSV glycoprotein D sequence revealed two antigenic determinants for T cells: one localized between residues 1–16 and the other between residues 8–23. The 1–16 site, which is helical, was recognized in the context of the Ia molecule, whereas the 8–23 site, which is nonhelical, was recognized in the context of the I-E molecule. The I-E–restricted response was found to be highly MHC degenerate in that T cell hybridomas specific for the 8–23 peptide responded to antigen on APCs derived from B10.A, B10.A(5R), and B10.A(9R) mice and showed differences in antigenic fine specificity with APCs of different haplotypes. These data support the idea of antigen–Ia interaction.

References

1. Blachandran, N., D. Harnish, W. E. Rawls, and S. Bachetti. 1982. Glycoproteins of herpes simplex virus type 2 as defined by monoclonal antibodies. *J. Virol.* 44:344.
2. Spear, P. 1984. Glycoproteins specified by herpes simplex viruses. *In* Herpesviruses. Vol. 3. B. Roizman, editor. Plenum Publishing Corp., New York. 315.
3. Chan, W. 1983. Protective immunization of mice with specific HSV-1 glycoproteins. *Immunology.* 49:343.
4. Paoletti, E., B. R. Lipinskas, C. Samsonoff, S. Mercer, and N. Panicali. 1984.

Construction of live vaccines using genetically engineered poxviruses: biological activity of vaccinia virus recombinants expressing hepatitis B virus surface antigen and the herpes simplex glycoprotein D. *Proc. Natl. Acad. Sci. USA.* 81:193.

5. Lasky, L. A., D. Dowbenko, C. C. Simonsen, and P. W. Berman. 1984. Protection of mice from a lethal herpes simplex virus infection by vaccination with a secreted form of cloned glycoprotein D. *Biotechnology.* 2:527.

6. Cremer, K., H. Mackett, C. Wohlenberg, A. Notkins, and B. Moss. 1985. Vaccinia virus recombinant expressing herpes simplex virus type 1 glycoprotein D prevents latent herpes in mice. *Science (Wash. DC).* 228:737.

7. Long, D., T. J. Madeira, M. Ponce de Leon, G. H. Cohen, P. C. Montgomery, and R. J. Eisendberg. 1984. Glycoprotein D protects mice against a lethal challenge with herpes simplex virus types 1 and 2. *Infect. Immun.* 37:761.

8. Cohen, G. H., B. Dietzschold, M. Ponce de Leon, D. Long, E. Golub, A. Varrichio, L. Periera, and R. J. Eisenberg. 1984. Localization and synthesis of an antigenic determinant of herpes simplex virus glycoprotein D that stimulates production of neutralizing antibody. *J. Virol.* 49:102.

9. Dietzschold, B., R. J. Eisenberg, M. Ponce de Leon, E. Golub, F. Hudecz, A. Varrichio, and G. H. Cohen. 1984. Fine structure analysis of type-specific and type-common antigenic sites of herpes simplex virus glycoprotein D. *J. Virol.* 52:431.

10. Heber-Katz, E., M. Hollosi, B. Dietzschold, F. Hudecz, and G. D. Fasman. 1985. The T cell response to the glycoprotein D of the Herpes Simplex Virus: significance of antigen conformation. *J. Immunol.* 135:1385.

11. Lamb, J. R., and N. Green. 1983. Analysis of the antigen specificity of influenza hemagglutinin-immune human T lymphocyte clones: identification of an immunodominant region for T-cells. *Immunology.* 50:659.

12. Thomas, J. W., D. E. Bullesbach, and A. S. Rosenthal. 1981. Immune response gene control of determinant selection. III. Polypeptide fragments of insulin are differentially recognized by T but not by B cells in insulin immune guinea pigs. *J. Immunol.* 126:1095.

13. Maizels, R. M., J. A. Clarke, M. A. Harvey, A. Miller, and E. E. Sercarz. 1980. Epitope specificity of the T-cell proliferative response to lysozyme: proliferative T-cells react predominantly to different determinants from those recognized by B cells. *Eur. J. Immunol.* 10:509.

14. Corradin, G., and J. M. Chiller. 1979. Lymphocyte specificity to protein antigens. II. Fine specificity of T-cell activation with cytochrome C and derived peptides as antigenic probes. *J. Exp. Med.* 149:436.

15. Berkower, L., L. A. Matis, G. K. Buckenmeyer, F. R. N. Gurd, D. L. Longo, and J. A. Berzofsky. 1984. Identification of distinct predominant epitopes recognized by myoglobin-specific T cells under the control of different Ir genes and characterization of representative T cell clones. *J. Immunol.* 132:1370.

16. Hurwitz, J. L., E. Heber-Katz, C. J. Hackett, and W. Gerhard. 1984. Characterization of the murine T$_H$ response to influenza virus hemagglutinin: evidence for three major specificities. *J. Immunol.* 133:3371.

17. Heber-Katz, E., R. H. Schwartz, L. A. Matis, C. Hannum, L. A. C. Hannum, T. Fairwell, E. Appella, and D. Hansburg. 1982. Contribution of antigen-presenting cell major histocompatibility gene products to the specificity of antigen-induced T cell activation. *J. Exp. Med.* 155:1086.

18. Heber-Katz, E., D. Hansburg, and R. S. Schwartz. 1983. The Ia molecule of the antigen-presenting cell plays a critical role in immune response gene regulation of T cell activation. *J. Mol. Cell. Immunol.* 1:3.

19. Werdlin, O. 1982. Chemically related antigens compete for presentation by accessory cells to T cells. *J. Immunol.* 129:1883.

20. Rock, K. L., and B. Benacerraf. 1983. Inhibition of antigenic-specific T lymphocyte activation by structurally related Ir-gene controlled polymers. Evidence for specific competition for accessory cell antigen presentation. *J. Exp. Med.* 155:1086.

21. Babbitt, B. P., P. M. Allen, G. Matsueda, E. Haber, and E. R. Unanue. 1985. Binding of immunogenic peptides to Ia histocompatibility molecules. *Nature* (Lond.). 317:359.

22. Watts, T. H., H. E. Gaub, and H. M. McConnell. 1986. T cell mediated association of peptide antigen and major histocompatibility complex protein detected by energy transfer in an evanescent wave-field. *Nature (Lond.).* 320:179.

23. Buus, S., A. Sette, S. Colon, C. Miles, and H. M. Grey. 1987. The relation between MHC restriction and the capacity of Ia to bind immunogenic peptides. *Science (Wash. DC).* 235:1353.

24. Phillips, M. L., C. C. Yip, E. M. Shevach, and T. L. Delovitch. 1986. Photo affinity labeling demonstrates binding between Ia molecules and nominal antigen on antigen-presenting cells. *Proc. Natl. Acad. Sci. USA.* 83:5634.

25. Griffith, I. J., E. M. Choi, and L. H. Glimcher. 1987. A single base mutation in an I-A alpha chain gene alters T cell recognition. *Proc. Natl. Acad. Sci. USA.* 84:1090.

26. Allen, P. M., G. R. Matsueda, R. J. Evans, J. B. Dunbar, Jr., G. R. Marshall, and E. R. Unanue. 1987. Identification of the T cell and Ia contact residues of a T cell antigenic epitope. *Nature (Lond.).* 327:713.

27. Ronchese F., R. H. Schwartz, and R. N. Germain. 1987. Functionally distinct subsites on a class II major histocompatibility complex molecule. *Nature (Lond.).* 329:254.

28. Merrifield, R. B. 1963. Solid phase peptide synthesis. I. The synthesis of a tetrapeptide. *J. Am. Chem. Soc.* 85:2149.

29. Julius, M. H., E. Simpson, and L. A. Herzenberg. 1973. A rapid method for the isolation of functional thymus-derived murine lymphocytes. *Eur. J. Immunol.* 3:645.

30. Gillis, S., M. M. Ferm, W. Ou, and K. A. Smith. 1978. T cell growth factor: parameters of production and a quantitative microassay for activity. *J. Immunol.* 120:2027.

31. Ozata, K., N. Mayer, and D. H. Sachs. 1980. Hybridoma cell lines secreting monoclonal antibodies to mouse H-2 and Ia antigens. *J. Immunol.* 124:533.

32. Oi, V. T., P. P. Jones, J. W. Goding, and L. A. Herzenberg. 1978. Properties of monoclonal antibodies to mouse Ig allotypes, H-2, and Ia antigens. *Curr. Top. Microbiol. Immunol.* 81:115.

33. Chou, P. W., and G. W. Fasman. 1978. The prediction of secondary structure of proteins from their amino acid sequence. *Adv. Enzymol. Relat. Areas Mol. Biol.* 47:45.

34. Matko, M., Z. Nagy, U. Koszinowski, and J. Klein. 1982. Involvement of MHc loci in immune responses that are not Ir-gene-controlled. *Immunogenetics.* 16:471–483.

35. Katz, D. H., T. Hamaoka, and B. Benacerraf. 1973. Cell interactions between histoincompatible T and B lymphocytes. Failure of physiologic cooperative interactions between T and B lymphocytes from allogeneic donor strains in the humoral response to hapten-protein conjugates. *J. Exp. Med.* 137:1405.

36. Rosenthal, A. S., and E. M. Shevach. 1973. Function of macrophages in antigen recognition by guinea pig T lymphocytes. I. Requirement for histocompatible macrophages and lymphocytes. *J. Exp. Med.* 138:1194.

37. Zinkernagel, R. M., and P. C. Doherty. 1979. MHC-restricted cytotoxic T cells: studies on the biological role of polymorphic major transplantation antigens determining T cell restriction specificity, function, and responsiveness. *Adv. Immunol.* 27:52.

38. Mengel-Gaw, L., and H. O. McDevitt. 1983. Isolation and characterization of cDNA clone for the murine E beta polypeptide chain. *Proc. Natl. Acad. Sci. USA.* 80:7621.

39. Mengel-Gaw, L., and H. O. McDevitt. 1985. Predicted protein sequence of the murine I-E beta s polypeptide chain from cDNA and genomic clones. *Proc. Natl. Acad. Sci. USA.* 82:2910.

40. Janeway, C., E. A. Lerner, P. Conrad, and B. Jones. 1982. The precision of self and nonself major histocompatibility complex encoded antigen recognition by cloned T cells. *Behring Inst. Mitt.* 70:200.

41. Doherty, P. C., and J. R. Bennick. 1979. Vaccinia-specific cytotoxic T cell responses in the context of H-2 antigens not encountered in the thymus may reflect abberant recognition of the virus-H-2 complex. *J. Exp. Med.* 149:150.

42. Hunig, T. R., and M. J. Bevan. 1982. Antigen recognition by cloned cytotoxic T lymphocytes follows rules predicted by the altered-self hypothesis. *J. Exp. Med.* 155:111.

43. Suzuki, G., and R. H. Schwartz. 1986. The pigeon cytochrome c specific T cell response of low responder mice. I. Identification of antigenic determinants on fragment 1 to 65. *J. Immunol.* 136:230.

44. Finnegan, A., M. A. Smith, J. A. Smith, J. Berzofsky, D. H. Sachs, and R. J. Hodes. 1986. The T cell repertoire for recognition of a phylogenetically distant protein antigen. *J. Exp. Med.* 164:899.

45. Matis, L. A., D. L. Longo, S. Hedrick, C. Hannum, E. Margoliash, and R. H. Schwartz. 1983. Clonal analysis of MHC restriction and fine specificity of antigen recognition in the T cell proliferative response to cytochrome c. *J. Immunol.* 130:1527.

46. Hedrick, S., L. A. Matis, T. T. Hecht, L. E. Samuelson, D. L. Longo, E. Heber-Katz, and R. H. Schwartz. 1982. The fine specificity of antigen and Ia determinant recognition by T cell hybridoma clones specific for pigeon cytochrome c. *Cell.* 30:141.

47. Zinkernagel, R. M., G. N. Callahan, A. Althage, S. Cooper, P. A. Klein, and J. Klein. 1978. Thymus in differentiation of "H-2 self recognition" by T cells: evidence for dual recognition. *J. Exp. Med.* 144:933.

48. Kapp, J. A., C. W. Pierce, S. Schlossman, and B. Benacerraf. 1974. Genetic control of the immune response in vitro. V. Stimulation of suppressor T cells in nonresponder mice by the terpolymer GAT. *J. Exp. Med.* 140:648.

49. Kotzin, B. L., V. L. Barr, and E. Palmer. 1986. A large deletion within the T-cell receptor beta-chain gene complex in New Zealand white mice. *Science (Wash. DC).* 229:167.

50. Rosenthal, A. S. 1978. Determinant selection and macrophage function in genetic control of the immune response. *Immunol. Rev.* 40:136.

51. Benacerraf, B. 1978. A hypothesis to relate the specificity of T lymphocytes and the activity of I region-specific Ir genes in macrophages and B lymphocytes. *J. Immunol.* 120:1809.

52. Hansburg, D., T. Fairwell, R. H. Schwartz, and E. Appella. 1983. The T lymphocyte response to cytochrome c. IV. Distinguishable sites on a peptide antigen which affect antigenic strength and memory. *J. Immunol.* 131:319.