Brief Definitive Report

LACK OF IMMUNODOMINANCE IN THE T CELL RESPONSE TO HERPES SIMPLEX VIRUS GLYCOPROTEIN D AFTER ADMINISTRATION OF INFECTIOUS VIRUS

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T cell responses to a single foreign protein antigen are generally specific for a few, and often only one, of the peptides derived by processing of that antigen (1, 2) and those determinants are referred to as immunodominant T cell epitopes. Though the mechanisms underlying such restricted T cell responses are unclear, several have been suggested: (*a*) availability of an appropriate peptide after antigen processing (1, 3), (*b*) the ability of a particular peptide to compete with other peptides for binding sites on the Ia molecule (3), and (*c*) the limitation of the expressed T cell repertoire during thymic development (1, 2).

We have explored in the present study the T cell response of H-2^d mice to glycoprotein D (gD), a coat protein of HSV, previously shown to confer a potent and long-lived protective immune response (4–6). Using a set of 28 overlapping synthetic gD peptides, we have examined T cell response patterns in mice immunized with recombinant gD-1 (a molecule lacking the transmembrane and cytoplasmic regions found in native gD-1) in CFA. Not unexpectedly, such T cells could be stimulated by only *one* of these 28 peptides (peptide 13: residues 241–260). We have also examined T cell responses to rgD-1 and to the gD peptides when mice were infected with HSV-1 in PBS. To our surprise, the pattern of responsiveness was radically different. There appeared to be no immunodominance displayed since at least 17 peptides (and up to 22 peptides) were found to be stimulatory. We believe these results call into question the validity of the concept of immunodominant determinants when considering immunity to infectious disease, and directly bear on issues of vaccine design.

Materials and Methods

Mice. B10.D2 mice were from The Jackson Laboratory (Bar Harbor, ME) and BALB/c mice from Charles River Breeding Laboratories Inc. (Wilmington, MA).

Antigens. rgD-1 was a generous gift from Dr. R. L. Burke (Chiron Corp., Emeryville, CA) and made as described elsewhere (7). Briefly, a truncated gD gene of HSV-1, strain Patton, was cloned in the plasmid PHS118 and used to transfect Chinese hamster ovary (CHO) cells. The rgD-1 is secreted as a glycosylated, 290 amino acid long molecule lacking 54 amino acids at the COOH terminus and transmembrane anchor domain. Immunoadsorbant-purified rgD-1 was used for immunization. A supernatant of transfected CHO cells was used for in vitro

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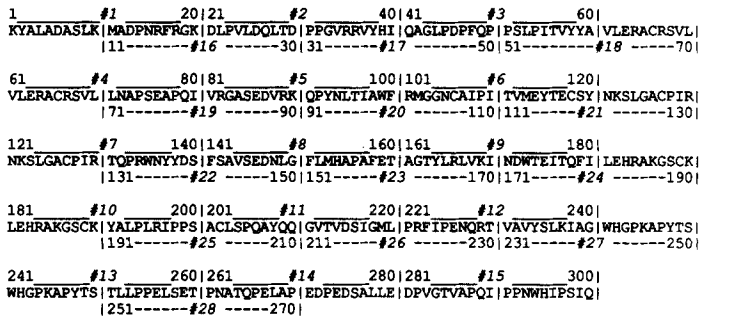

FIGURE 1. Amino acid sequences of recombinant gD-1 and of its overlapping peptides.

T cell stimulation. Synthetic peptides were either purchased from Biosearch (San Rafael, CA) or synthesized as described (6). All peptides are 20-mers and span the entire length of rgD-1 with a set of peptides having 10 amino acid overlaps on either side (Fig. 1).

Virus Preparation. HSV-1, strain (F), was provided by Dr. Nigel Fraser (The Wistar Institute). The virus was grown, and its titer was measured in BS-C-1 cells.

Lymph Node T Cell Proliferation Assay. Mice were immunized subcutaneously in the hind footpads with 2.0 μg of rgD-1 in CFA or 5×10^5 PFU of HSV-1 (F) in 20 μl of PBS. 7 d later, cells from draining lymph nodes were treated with J11D + complement. T cells ($2-5 \times 10^5$) were cultured in 96-well microtiter plates with $1-3 \times 10^5$ X-irradiated (2,000 rad) normal spleen cells with or without antigen. After 4 d, the degree of proliferation was determined as [^{3}H]thymidine incorporation into DNA.

Antibody Blocking Experiments. Various concentrations of ascitic fluid or ammonium sulfate-precipitated culture supernatant of mAb MKD6 (anti-I-A^d) or 14-4-4S (anti-I-E) were added at the initiation of culture. The percent inhibition was calculated as: $100 \times (1 -$ experimental response/control response).

T Cell Lines. B cell-depleted lymph node cells (5×10^6/ml) from HSV-primed mice were cultured with X-irradiated syngeneic spleen cells (2,000 rad, 5×10^6/ml) plus antigen for 10 d. Viable cells (5×10^5/ml) were restimulated with antigen plus 5×10^6/ml of X-irradiated (2,000 rad) splenocytes. Antigen specificity was analyzed after four cycles of stimulation.

Results and Discussion

Adult BALB/c and B10.D2 mice were immunized with rgD-1 in CFA and their lymph node (LN) T cells were stimulated in vitro with the described set of overlapping synthetic gD peptides (Fig. 2, *A* and *B*). T cells from both strains of mice responded exclusively to peptide 13 (residues 241–260), which seems to be the only immunodominant gD determinant in H-2^d mice. The remaining peptides elicited no significant responses. These findings are in accordance with the report of Chestnut et al. (8) who found residues 245–260 to be the BALB/c immunodominant determinant of gD.

To explore immunodominance in the context of a natural infection, we challenged BALB/c mice subcutaneously with 5×10^5 PFU of HSV-1 (F) in PBS in the hind footpads. At this challenge dose of virus, none of the mice showed signs of neurological disease up to 2 mo after innoculation. The proliferative response of T cells from these HSV-primed animals either to HSV or to gD was at its peak around day 6–7 after viral innoculation and declined thereafter (data not shown). 7 d after challenge, the antigen specificity of these T cells was analyzed (Fig. 2 *C*).

Surprisingly, virus-primed LN T cells were found to respond not only to peptide

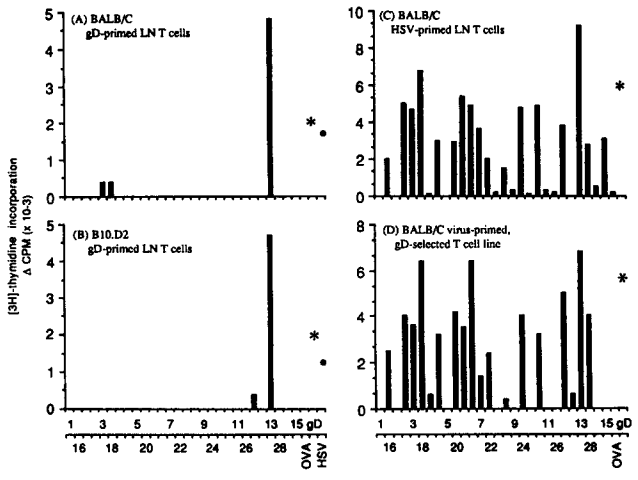

FIGURE 2. (*A* and *B*). Proliferative responses of rgD-1-primed LN T cell from BALB/c and B10.D2 mice. LN T cells (3×10^5) were cultured with X-irradiated syngeneic spleen cells (10^5), together with various antigens including synthetic gD peptides (10 μg/ml), OVA (10 μg/ml), UV-HSV (10^6 PFU/ml), and rgD-1 (1 μg/ml). Cells were cultured for 4 d and then pulsed with [^{3}H]thymidine. Background-subtracted geometric means are shown. Background thymidine incorporation without antigen was, in both cases, <2,000 cpm. (*C*) Proliferative responses of LN T cells from HSV-infected BALB/c mice. T cells (5×10^5) were cultured as described in *A*. Background incorporation was 1,800 cpm. (*D*) A rgD-1-selected T cell line from HSV-infected BALB/c mice. 10^4 gD-selected T cells were cultured as described in *A*. The background incorporation without antigen was 2,000 cpm.

13 but also to the majority of the other peptides (i.e., a total of 17 of 28). Though not shown, the response to gD was approximately one-third of the response to 10^6 PFU/ml of UV-inactivated HSV (25,000 cpm). While the responses to these 17 peptides were reproducibly observed in all four experiments performed, occasional but significant responses to other peptides, such as peptides 2, 4, 5, 8, and 11, were also detected at least once.

Considering the fact that HSV carries many other antigens, it could be argued that those T cells reactive to peptides other than peptide 13 are, in reality, specific for other HSV-related antigens that are crossreactive to the gD peptides used. Another explanation is that these peptide-reactive T cells are specific for gD and that a viral challenge caused a response very different from that elicited by immunization with a protein molecule in CFA.

To examine the gD specificity of those peptide-reactive T cells, we stimulated infectious virus-primed T cells with either rgD-1 or gD peptides to establish long-term T cell lines. Fig. 2 *D* shows that a long-term, gD-selected line maintained almost the same antigen specificity pattern as that of the primary T cells (Fig. 2 *C*). Furthermore, peptide-selected lines were shown to respond to rgD-1 as well as to peptide (Table I). Thus, peptide-reactive T cells found in infectious virus-primed LN T cells do contain gD-specific cells.

The fine specificity of the peptide-selected T cell lines was further dissected using anti-Ia antibodies (Table II). Anti-I-A^d mAb, MKD6, blocked the responses of peptide 13- and 25-selected T cell lines to rgD-1, whereas anti-I-E mAb, 14-4-4S, did not. Thus, these responses are completely I-A restricted. On the other hand, the responses of peptide 17- and 18-selected T cell lines to rgD-1 were significantly blocked by both MKD6 (49 and 67%, respectively) and 14-4-4S (34 and 31%, respectively), indicating the existence of heterogeneous gD-specific T cell populations, ones that are I-A restricted and others that are I-E restricted.

TABLE I

The Proliferative Responses of HSV-primed Peptide-selected T Cell Lines

	Proliferative activity* of T cell lines selected with:								
Antigen	No. 16 11-30	No. 17 21-40	No. 18 51-70	No. 19 71-90	No.21 111-130	No. 24 171-190	No. 25 191-210	No.12 221-240	No. 13 241-260
	cpm × 10^{-3} ± SD								
Medium	1.3 ± 0.2	1.2 ± 0.3	2.8 ± 0.3	1.6 ± 0.4	1.7 ± 0.4	1.9 ± 0.6	2.1 ± 0.9	2.8 ± 0.4	4.0 ± 0.8
Peptide	4.8 ± 0.6	5.7 ± 0.8	9.0 ± 0.7	4.5 ± 0.8	9.8 ± 1.0	7.9 ± 0.8	8.7 ± 1.0	10.3 ± 0.9	18.0 ± 1.9
rgD-1	5.2 ± 0.4	5.9 ± 0.9	10.3 ± 1.0	5.3 ± 0.9	8.9 ± 1.1	8.3 ± 1.4	10.5 ± 1.2	11.6 ± 1.3	16.0 ± 1.6
68-88‡	1.4 ± 0.5	1.4 ± 0.1	2.7 ± 0.8	1.2 ± 0.3	1.6 ± 0.3	2.1 ± 0.9	2.0 ± 0.3	2.6 ± 0.6	2.9 ± 0.3

* Peptide-selected BALB/c T cells (10^4) were cultured with 10^5 irradiated spleen cells with 2 μg/ml of gD peptide, 1μg/ml of rgD-1, or 10 μg/ml of irrelevant peptide. Cells were incubated for 2.5 d. Results are mean [^{3}H]thymidine incorporation in cpm ± SD for duplicate cultures.

‡ A synthetic peptide from guinea pig myelin basic protein (residues 68-88).

TABLE II

Anti-Ia Antibodies Block gD-specific Responses of Peptide-selected T Cells

	Proliferative activity*							
	No. 13 (241-260)		No. 25 (191-21)		No. 17 (31-50)		No. 18 (51-70)	
mAb	Med‡	gD-1	Med	gD-1	Med	gD-1	Med	gD-1
None	1.2 ± 0.2	15.0 ± 0.8	1.0 ± 0.2	10.4 ± 1.0	0.9 ± 0.0	6.6 ± 0.8	1.0 ± 0.0	6.5 ± 0.1
Anti-IA	0.6 ± 0.3	4.5 ± 0.6 (73)§	1.2 ± 0.1	2.6 ± 0.4 (89)	1.4 ± 0.2	4.2 ± 0.5 (49)	1.3 ± 0.1	3.6 ± 0.1 (67)
Anti-IE	0.9 ± 0.1	14.1 ± 0.9 (5)	1.6 ± 0.3	11.1 ± 0.7 (−1)	1.0 ± 0.2	4.8 ± 0.3 (34)	1.2 ± 0.2	5.0 ± 0.1 (31)

* HSV-primed, petide-selected T cells (10^4) were cultured for 2.5 d with 10^5 irradiated splenocytes with 1 μg/ml of rgD-1 (gD-1). Anti-IA mAb, MKD6, and anti-IE mAb, 14-4-4S, were added at the beginning of culture at a final concentration of 0.3%. The results are duplicate cultures with a mean [^{3}H]thymidine incorporation.

‡ Med, medium control.

§ The numbers in the parentheses indicate the percent inhibition of the response.

Taken together, these results indicate extensive diversity and a corresponding lack of immunodominance in the T cell response to gD in H-2^d mice when gD-specific T cells are recruited during the acute phase of an HSV infection. There are several possibilities that might explain the difference between gD-specific responses elicited by infectious virus versus rgD in CFA. (*a*) There are structural differences between viral gD and the recombinant gD used in these studies. A transmembrane and/or a cytoplasmic portion of the gD molecule that is lacking in rgD-1 might be involved in facilitating presentation of some of the "nonimmunodominant" gD determinants in virus-primed animals. (*b*) There are potential differences in the in vivo concentration of gD. Although a 20-fold increase of a challenge dose of rgD-1 did not render "nonimmunodominant" T cell populations detectable in a primary proliferation assay (data not shown), the concentration of gD provided by immunization with CFA may not be as high as that achieved in a virus infection that does induce a full-range,

gD-specific T cell response. (*c*) There is a possible difference in exogenous vs. endogenous antigen processing and presentation. Some gD determinants might be more efficiently presented by virus-infected APCs as seen in the influenza neuraminidase system (9). (*d*) There could be differences in the level of Ia expression on APCs. Due to intense inflammatory responses elicited by viral infection, the level of Ia expression on APCs could be upregulated, rendering them more potent APCs (10). (*e*) There may be quantitative differences in the APCs used in vivo. B cells are proposed to function as APCs in clonal expansion of T cells, especially in lymph nodes (11). In the case of a viral infection, not only gD-specific B cells but also B cells specific for any of the HSV antigens could function as APC to expand gD-specific T cells. The factors mentioned above might operate alone or together in a positive fashion to induce the T cell response seen during an acute HSV infection.

We were at first surprised that neither rgD-1 nor infectious virus induced a response to the 1-20 peptide since we had already shown that the 1-23 peptide coupled to palmitic acid and incorporated into a liposome conferred potent long-term T cell-mediated protection against a lethal HSV challenge (6). However, we note that depletion of $CD8^+$ cells could eliminate adoptive transfer of protection while the response measured in the present study is mediated by $CD4^+$ T cells (data not shown). Furthermore, the protection-conferring determinant on the 1-23 construct lies near the COOH terminus as an 8-23 construct protects and a 1-16 construct does not (Yamashita, K., and E. Heber-Katz, manuscript in preparation). Also, the fact that the 11-30 peptide (No. 16) was stimulatory after priming with infectious virus supports the importance of this COOH-terminal region. Finally, these studies emphasize that immunization protocols involving infectious virus versus rgD in CFA versus peptide-lipid-liposome constructs in CFA are not equivalent in the qualitative nature of the immunity induced and bear on issues of vaccine design.

Extensive diversity in T cell responses has been previously seen with influenza hemagglutinin (12, 13). That the T cells analyzed were derived from virus-infected animals in both of these studies, plus our own, suggests that T cell response patterns observed when animals are immunized with a purified protein in adjuvant do not necessarily reflect the maximum immune potential of an animal. Though T cell immunodominance has been demonstrated, even in the case of viral infections (here $CD8^+$ T cells were examined; reference 14), the results presented here raise the issue of T cell immunodominance generally as a biologically meaningful concept.

Summary

Glycoprotein D (gD) of HSV has been shown to be a potent immunogen. To analyze the T cell antigenic determinants on gD, a series of 28 overlapping 20-mer peptides that span the extracellular portion of gD-1 were examined for their ability to stimulate T cells from rgD-1 or infectious HSV-1-primed H-2^d mice in vitro. rgD-1-primed cells responded exclusively to peptide 241-260, the immunodominant determinant of gD in H-2^d mice. In contrast, infectious HSV-primed T cells were shown to respond to 17 (and up to 22) of 28 synthetic gD peptides. These results indicate an extensive diversity in the T cell repertoire to gD in H-2^d mice with T cells directed to a broad array of peptide determinants being recruited during the acute phase of an HSV infection.

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