JOURNAL OF VIROLOGY, May 1994, p. 2803–2810
0022-538X/94/$04.00+0

Vol. 68, No. 5

Antigenic Specificities of Human CD4+ T-Cell Clones Recovered from Recurrent Genital Herpes Simplex Virus Type 2 Lesions

DAVID M. KOELLE,[1]* LAWRENCE COREY,[1] RAE LYN BURKE,[2] ROSELYN J. EISENBERG,[3] GARY H. COHEN,[3] RATH PICHYANGKURA,[4] AND STEVEN J. TRIEZENBERG[4]

Departments of Medicine and Laboratory Medicine, University of Washington, Seattle, Washington 98195[1]; Chiron Corporation, Emeryville, California 94608[2]; University of Pennsylvania, Philadelphia, Pennsylvania 19104[3]; and Michigan State University, East Lansing, Michigan 48824[4]

Received 22 November 1993/Accepted 24 January 1994

Lesions resulting from recurrent genital herpes simplex virus (HSV) infection are characterized by infiltration of CD4+ lymphocytes. We have investigated the antigenic specificity of 47 HSV-specific CD4+ T-cell clones recovered from the HSV-2 buttock and thigh lesions of five patients. Clones with proliferative responses to recombinant truncated glycoprotein B (gB) or gD of HSV-2 or purified natural gC of HSV-2 comprised a minority of the total number of HSV-specific clones isolated from lesions. The gC2- and gD2-specific CD4+ clones had cytotoxic activity. The approximate locations of the HSV-2 genes encoding HSV-2 type-specific CD4+ antigens have been determined by using HSV-1 × HSV-2 intertypic recombinant virus and include the approximate map regions 0.30 to 0.46, 0.59 to 0.67, 0.67 to 0.73, and 0.82 to 1.0 map units. The antigenic specificity of an HLA DQ2-restricted, HSV-2 type-specific T-cell clone was mapped to amino acids 425 to 444 of VP16 of HSV-2 by sequential use of an intertypic recombinant virus containing VP16 of HSV-2 in an HSV-1 background, recombinant VP16 fusion proteins, and synthetic peptides. Each of the remaining four patients also yielded at least one type-specific T-cell clone reactive with an HSV-2 epitope mapping to approximately 0.67 to 0.73 map units. The antigenic specificities of lesion-derived CD4+ T-cell clones are quite diverse and include at least 10 epitopes. Human T-cell clones reactive with gC and VP16 are reported here for the first time.

Recurrent herpes simplex virus (HSV) infection in humans is a localized cutaneous disease in individuals with intact cellular immunity. The roles of infiltrating cells in host defense are incompletely understood. The cellular infiltrate is enriched for CD4+ cells during early stages of lesion formation (13). In murine models of HSV infection, adoptively transferred bulk (28) or cloned (24) CD4+ T cells can protect against viral challenge. Keratinocytes, the predominant infected cell type in HSV skin lesions, are capable of presenting HSV antigen to CD4+ T cells after gamma interferon treatment (12). Functional roles of lesion-infiltrating CD4+ T cells in recurrent HSV lesions may include lymphokine release, inhibition of viral replication, cytotoxicity, and B-cell help (48–50).

The antigens recognized by human HSV-specific CD4+ T cells have not been fully characterized. The membrane glycoproteins B, C, and D (gB, gC, and gD) stimulate proliferation of T lymphocytes from peripheral blood mononuclear cells (PBMC) of HSV-seropositive individuals (43, 52). Secondary in vitro stimulation of PBMC with HSV antigen has been required to expand and enrich HSV-specific T cells prior to derivation of HSV-specific T-cell clones (51). A large proportion of the T-cell clones isolated and maintained by periodic restimulation with whole HSV antigen were reactive with gB or gD (52), and a substantial proportion of these clones also had cytotoxic T-lymphocyte (CTL) activity (48). It is not known whether secondary in vitro stimulation with crude viral antigen influences the relative proportions of resultant clones reactive with specific HSV proteins or displaying CTL or other effector functions.

CD4+ T cells recognize primarily exogenously synthesized antigen after processing by appropriate antigen-processing cells (7). Abundant virion proteins are thus candidates for CD4+ T-cell antigens. Strong serum antibody responses to viral glycoproteins and the abundant tegument protein VP16 are present in human HSV-1 and HSV-2 infections (1), consistent with the presence of a CD4+ helper T-cell response. However, the presence of a human CD4+ response to VP16 has not been previously demonstrated.

We have recently developed techniques for isolating HSV-specific CD4+ T-cell clones from recurrent human HSV lesions (23). We report here the identification of the antigens recognized by a subset of these T-cell clones and the mapping of additional HSV-2 type-specific CD4+ T-cell antigens with the use of HSV-1 × HSV-2 intertypic recombinant viruses (IRV).

MATERIALS AND METHODS

Viruses. HSV-2 strain 333 (22) and HSV-1 strain E115 (40) were used throughout unless otherwise specified. HSV-1 × HSV-2 IRV RH1G7, RS1G25, RS1G31, and R7015 (31, 32) were the kind gift of Bernard Roizman. HSV-1 strain MP801-1, expressing the gene for gC of HSV-2, and HSV-2 strain G_PS1, containing a deletion of gC, were the kind gift of Patricia Spear (53). An IRV (designated RP-2) bearing the VP16 gene from HSV-2 (strain HG52) in the HSV-1 (KOS) genome was constructed by complementation of the VP16-deleted virus 8MA (45). The HSV-2 fragment includes the entire VP16 open reading frame with 30 bp of 5′ flanking sequence and 43 bp of 3′ flanking sequence.

* Corresponding author. Mailing address: Pacific Medical Center, Room 9301, 1200 12th Ave. S., Seattle, WA 98144. Phone: (206) 326-4162 (laboratory); (206) 326-4177 (office). Fax: (206) 326-4178. Electronic mail address: viralimm@u.washington.edu.

Virus stocks were prepared by infecting HDF cells at a multiplicity of infection of 0.001 to 0.01 or 1.0 (strain RP-2 only) and allowing the infection to progress until cytopathic effect involved 80 to 100% of the cells. Scraped cells were sonicated and then centrifuged at 400 × *g* for 10 min after addition of 2 ml of minimal essential medium–10% fetal calf serum per original 150-cm^2 flask of HDF cells. Supernatant was frozen in aliquots, and virus titers were determined by a plaque assay on Vero cells (37). Titers ranged from 10^7 to 3 × 10^9 PFU/ml. Mock virus preparations were made from the same batch of HDF. Recombinant vaccinia viruses containing the genes for gB and gD of HSV-2 (42) and wild-type vaccinia virus New York were grown on HDF and prepared in the same fashion as HSV strains, and titers were determined by a plaque assay on BHK-21 cells (37).

Cell lines. PBMC were prepared by Ficoll-Hypaque density gradient centrifugation and cryopreserved for use as antigen-presenting cells (APC). Epstein-Barr virus-transformed lymphoblastoid cell lines (LCL) were prepared from PBMC as described previously (42) and maintained in RPMI-FC (RPMI 1640, 25 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid [HEPES], 2 mM L-glutamine, 50 μg of streptomycin per ml, 50 U of penicillin per ml, 2 × 10^{-5} M β-mercaptoethanol, 1 mM pyruvate, 10% fetal calf serum). HDF cells (37) were maintained in minimal essential medium–10% fetal calf serum. Cells used for preparation of viral stocks and functional assays were negative for mycoplasma (Mycotect; GIBCO, Grand Island, N.Y.).

T-lymphocyte culture. Lymphocytes recovered from swabs or biopsies of culture-positive HSV-2 lesions were cloned by using a high efficiency procedure with phytohemagglutinin (PHA) and interleukin 2 (IL-2) (27) as previously described (23). Briefly, vesicle fluid and scraped cells from vesicle bases, or cells recovered from 3-mm skin biopsies minced with scissors and expressed through a 190-μm-pore-size sieve (Cellector; Bellco Glass, Inc., Vineland, N.J.), were underlaid with an equal volume of Ficoll-Hypaque. Cells were recovered from the interface after centrifugation at 400 × *g* for 30 min at room temperature. These cells were 90 to 95% polymorphonuclear leukocytes, with occasional eosinophils and monocytes, 1% or less small round mononuclear cells, and 5 to 10% epithelial cells with or without multiple nuclei or inclusions. The cells were plated at 10 to 200 cells per well in T-cell medium (RPMI 1640 containing 25 mM HEPES, 2 mM L-glutamine, 50 μg of streptomycin per ml, 50 U of penicillin per ml, and 10% human blood type AB^+ serum) and stimulated with PHA, allogeneic irradiated PBMC as feeder cells, and 20 to 50 U of IL-2 (Pharmacia, Piscataway, N.J.) per ml. Acyclovir (50 μM) was included for the first 2 weeks to inhibit HSV replication. Between 7.9 and 48.7% of wells were positive for growth by microscopic examination. Clones were periodically restimulated with PHA, allogeneic feeder cells, and IL-2 (42). Subclones were derived at one cell per well (27).

In addition, cells (4.2 × 10^5) recovered from swabs from patient 1, lesion A, were stimulated in bulk with 10^6 allogeneic irradiated (3,300 rads of gamma irradiation) PBMC and 0.4 μg of PHA-P (Murex Diagnostics Ltd., Dartford, England) per ml in 2 ml of T-cell medium plus 50 μM acyclovir in a 1.88-cm^2 well. After 48 h, 1 ml of medium was exchanged for medium containing 50 U of IL-2 per ml. Cells were split as necessary and fed with T-cell medium containing 50 U of IL-2 per ml. Clones were derived after 19 days of growth by plating the cells at one cell per well with allogeneic irradiated PBMC, PHA, and IL-2 (27).

Proliferation assays. Screening assays for lesion-derived T-cell clones were performed in duplicate as described previously (23). Cloned T cells or bulk lesion-derived cells (10^4 per well) and antigen were added to a final volume of 200 μl of T-cell medium in 96-well U-bottom plates. Autologous gamma-irradiated (3,300 rads) PBMC (10^5 per well) were included as APC. After 3 days, [^{3}H]thymidine (1 μCi per well) was added for 18 h; cells were harvested with a semiautomated harvester and counted by liquid scintillation. All subsequent assays were performed in triplicate. Clones were judged to be specific for HSV strains or purified antigens if the stimulation index (cpm of [^{3}H]thymidine incorporation for HSV strain or antigen/cpm of [^{3}H]thymidine incorporation for mock HDF antigen) was greater than 4.0. Stimulation indices are reported with two significant figures to reflect the precision of the experimental measurements. For experiments with allogeneic APC, proliferation in response to both mock and HSV antigens was measured with allogeneic irradiated PBMC, and stimulation indices were calculated from these two measurements.

Antibody blocking of proliferation. Monoclonal antibodies (MAbs) L243 (38), B7/21 (35), and SPV-L3 (39) recognize HLA DR, DP, and DQ framework determinants, respectively. Supernatant of L243 cells, obtained from the American Type Culture Collection, was used at 1:4 (final dilution). Purified B7/21, obtained from Tom Cotner, was used at 1:400 (final dilution). Purified SPV-L3, obtained from Hans Yssel, was used at 10 μg/ml (final concentration). These concentrations were determined in preliminary titration experiments to inhibit proliferation of $CD4^+$ T-cell clones restricted by the relevant HLA class II loci by greater than 80% and to inhibit proliferation of T-cell clones restricted at irrelevant HLA class II loci by less than 15%.

Antigens. Antigens were prepared from viral stocks by exposure to UV light for 30 min at 10 cm from a new GT038 bulb, eliminating infectious virus. Antigens were routinely used in lymphoproliferation assays at 1:100 (final dilution), corresponding to 10^5 to 10^7 PFU/ml prior to UV treatment. Recombinant gB and gD of HSV-2 were purified from transfected Chinese hamster ovary cells (8). gB2 is truncated at amino acid 696, and gD2 is truncated at amino acid 302. Recombinant glycoproteins were used at 2 μg/ml (final concentration) (52). gC of HSV-2 was obtained by affinity chromatography of infected cells (43) and used at 2 μg/ml (final concentration). Fusion proteins containing the DNA-binding domain of the yeast protein GAL4 (amino acids 1 to 147) and the activation domains of VP16 (amino acids 413 to 490 or 402 to 490 for HSV-1 [36]) and HSV-2, respectively, were purified from *Escherichia coli* to greater than 95% homogeneity by selective precipitation and ion-exchange chromatography (37a). Peptides corresponding to amino acids 400 to 490 of VP16 of HSV-2, 15 amino acids long and overlapping by 10 amino acids (Chiron Mimotopes, Clayton, Australia), were dissolved in 20% acetonitrile–0.1 M acetic acid at 2.0 mg/ml and used at 10 μg/ml (final concentration).

Cytotoxicity assays. Target LCL were infected for 18 h with HSV or vaccinia viruses at a multiplicity of infection of 5, loaded with ^{51}Cr, and washed as previously described (42). Cloned lymphocytes were plated in 100 μl of RPMI-FC in 96-well U-bottom plates, and targets (5 × 10^3 per well) added in 100 μl of RPMI-FC. After 4 h at 37°C, the plates were centrifuged at 50 × *g* for 2 min, and 100 μl of supernatant was counted with a gamma counter. Allogeneic target cells were mismatched at HLA class I and II loci. Results are reported as percent specific release, determined as [(mean experimental cpm − mean spontaneous cpm)/(mean maximal cpm − mean spontaneous cpm)] × 100. Spontaneous release was always less than 20% of total release.

TABLE 1. HSV-specific CD4+ T-cell clones derived directly from HSV-2 lesions and available for antigenic specificity analysis

Patient, lesion	Serology		No. of HSV-specific CD4+ clones available for detailed study	
	HSV-1	HSV-2	Type specific[a]	Type common[b]
1, A	+	+	6[c]	1
1, B	+	+	1	2
2	−	+	2	1
3, B	+	+	2	10
4	−	+	8	4
5	−	+	7	3

[a] Stimulation index for HSV-2 antigen greater than 4.0.
[b] Stimulation indices for HSV-1 and HSV-2 antigens greater than 4.0.
[c] Includes five HSV-2 type-specific CD4+ T-cell clones derived from bulk culture of lesion-derived T lymphocytes (see text).

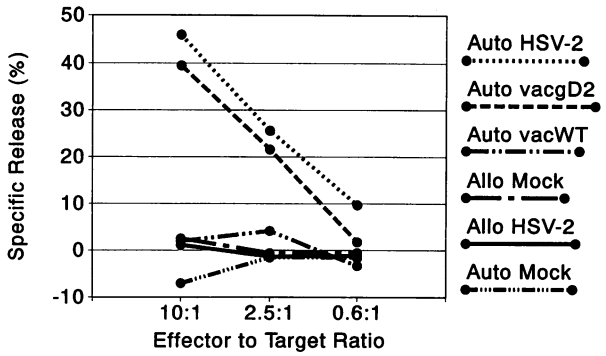

FIG. 1. Cytotoxic activity of lesion CD4+ T-cell clone 3B.134 against HSV-2- and vaccinia virus-infected and uninfected autologous (Auto) and allogeneic (Allo) LCL. Allogeneic LCL were mismatched at HLA class I and II loci.

Flow cytometry. T-cell clones were characterized for cell surface expression of CD3, CD4, and CD8 as previously described (23).

Serology. Type-specific serologies for HSV-1 and HSV-2 were performed by immunoblotting (2).

RESULTS

Cloning of lesion CD4+ HSV-specific T cells. In this report, we describe the antigenic specificities of 47 lesion-derived HSV-specific CD4+ T-cell clones from five patients (Table 1), representing all of the HSV-specific clones available in sufficient number for detailed study. A total of 42 HSV-specific CD4+ T-cell clones obtained directly from swabs or biopsies of recurrent HSV-2 buttock or thigh lesions were available in sufficient number for detailed studies. Details of the cloning procedure and reactivity with HSV-1 and HSV-2 antigens have been previously reported for patient 1, lesion A, and patients 2, 3, and 4 (23). In addition, five HSV-2 type-specific CD4+ T-cell clones, designated 1A.Bx, were obtained by cloning bulk lesion-derived cells from patient 1, lesion A, after 19 days of bulk culture. All clones were CD3+, CD4+, and CD8− by flow cytometry. Overall, 26 of 47 (55%) of the lesion-derived HSV-specific T-cell clones recognized HSV-2 type-specific epitopes.

gB- and gD-specific CD4+ T-cell clones. The HSV-specific CD4+ T-cell clones were each tested for reactivity with recombinant truncated gB and gD of HSV-2. Three clones from three different patients had stimulation indices of greater than 5.0 with gB2 (3B.268, 4.4D3, and 5.5), and one clone had a stimulation index of greater than 5.0 with gD2 (3B.134) (Table 2, experiment 1). Proliferation of clone 3B.134 was inhibited by greater than 90% by anti-HLA DP MAb but not by anti-HLA DR MAb (data not shown). The gD2-specific clone 3B.134 also lysed vaccinia virus-gD2- and HSV-2-infected autologous target cells, but not uninfected or wild-type vaccinia virus-infected autologous cells or HSV-2-infected allogeneic cells, in a cytotoxicity assay (Fig. 1). The gB2-specific clones did not have cytolytic activity against HSV-2- or vaccinia virus-gB2-infected target cells (data not shown). All four clones reactive with gB2 or gD2 recognized type-common determinants (Table 2, experiment 2). Subclones of each of the gB- and gD-reactive clones obtained at one cell per well retained the same pattern of reactivity with purified recombinant glycoproteins, HSV-1 antigens, and HSV-2 antigens (data not shown). The remaining T-cell clones all had stimulation indices with gB2 and gD2 of less than 3.0 (data not shown).

Epitope mapping using IRV. Fourteen of the 26 (54%) HSV-2 type-specific clones displayed stimulation indices of greater than 4.0 to at least one viral antigen prepared from a panel of IRV (Table 3). Twelve HSV-2 type-specific T-cell clones did not proliferate in response to antigen prepared from any of the IRV. Clone 4.3C12 is shown as an example (Table 3). All IRV elicited specific proliferative responses from bulk PBMC of HSV-seropositive individuals, demonstrating their antigenicity. As expected, T-cell clones reactive with type-common epitopes reacted with all IRV. Clone 1A.2 is shown as an example (Table 3). Data for the 20 additional type-common and 11 additional unmappable HSV-2 type-specific T-cell clones are not included in Table 3. The T-cell clones all displayed identical patterns of reactivity in at least two assays.

Type-specific T-cell clones recognized diverse HSV-2 antigens. Three HSV-2 type-specific clones (1A.1, 1B.9F8, and 5.4)

TABLE 2. Lesion-derived CD4+ clones specific for gB2 and gD2

Expt	Antigen	Mean [3H] incorporation (SD)[a]			
		3B.134	3B.268	4.4D3	5.5
1	Mock	344 (40)	448 (112)	839 (173)	107 (26)
	HSV-2	**6,173 (597)**	**24,749 (6,634)**	**24,195 (2,732)**	**19,954 (1,553)**
	gB2[b]	274 (40)	**24,595 (5,855)**	**61,062 (2,842)**	**35,545 (3,942)**
	gD2[b]	**5,454 (1,355)**	726 (133)	715 (88)	678 (573)
2	Mock	492 (105)	914 (145)	308 (80)	499 (188)
	HSV-1	**21,292 (2,911)**	**49,963 (2,670)**	**15,982 (117)**	**39,947 (1,554)**
	HSV-2	**44,290 (9,670)**	**72,997 (7,555)**	**19,456 (402)**	**36,867 (1,083)**

[a] Determined from triplicate samples. Glycoprotein-specific reactivity is indicated in boldface.
[b] Used at 2 μg/ml (final concentration).

TABLE 3. Proliferative responses of lesion-derived, HSV-2 type-specific CD4+ T-cell clones to antigens prepared from HSV-1, HSV-2, and HSV-1 × HSV-2 IRV

Responder	Stimulation index[a]					
	HSV-1 (0[b])	HSV-2 (0.0–1.0)	RH1G7 (0.30–0.46)	RS1G25 (0.59–0.73)	RS1G31 (0.67–0.73)	R7015 (0.82–1.0)
HSV-2 type-specific clones						
1A.1	1.0	4.9	**13**	1.8	ND	0.9
1A.B.25	0.9	79	0.9	98	**149**	1.0
1B.9F8	1.3	18	**20**	0.6	1.2	1.2
2.1	1.3	10	1.5	**22**	1.5	1.4
2.3	0.8	21	1.5	56	**57**	1.0
3B.230	1.3	8.5	2.0	**7.7**	1.8	0.9
3B.294	1.4	23	1.4	26	**24**	1.5
4.1A11	2.0	12	1.8	13	**13**	1.4
4.2E1	1.7	15	1.1	14	**11**	1.4
4.2F10	0.6	81	2.6	68	**103**	3.4
4.3C3	0.9	5.2	0.5	6.4	**6.8**	1.0
5.1	1.1	13	1.7	16	**11**	1.7
5.4	1.0	27	**25**	1.1	2.9	2.8
5.19	1.5	6.0	1.2	1.2	1.8	**6.6**
Controls						
PBMC[c]	11	21	15	17	13	11
1A.2[d]	25	15	20	29	24	28
4.3C12[e]	1.0	39	0.5	1.1	0.6	0.8

[a] Data defining specificity for an HSV-2 map region are indicated here and in Tables 4 and 5 in boldface. ND, not done.
[b] Approximate HSV-2 map region.
[c] Bulk nonirradiated PBMC (10^5) from an HSV-1- and HSV-2-seropositive donor were cultured with viral antigen at 1:100 (final dilution) and labeled with [^{3}H]thymidine from days 5 to 6.
[d] Representative clone recognizing a type-common epitope. Data for remaining type-common clones are not shown but are similar.
[e] Representative clone recognizing an HSV-2 type-specific epitope not included in the panel of IRV. Data for additional clones unreactive with the panel of IRV are not shown but are similar.

reacted with IRV RH1G7 (10, 32), corresponding to approximately 0.30 to 0.46 map units within the HSV-2 genome. Clones 1A.1 and 1B.9F8 were recovered from lesions from the same patient on opposite buttocks separated in time by 1 year. One HSV-2 type-specific clone (5.19) reactive with IRV R7015 (32), corresponding to approximately 0.82 to 1.0 map units within the HSV-2 genome, was isolated from patient 5. A total of 10 HSV-2 type-specific clones reacted with IRV RS1G25 (31, 32), corresponding to approximately 0.59 to 0.73 map units within the HSV-2 genome. Of these 10 clones, 8 (1A.B.25, 2.3, 3B.294, 4.1A11, 4.2E1, 4.2F10, 4.3C3, and 5.1) also reacted with IRV RS1G31 (31), indicating specificity for an epitope encoded by HSV-2 between approximately 0.67 and 0.73 map units. All eight clones reactive with RS1G31 were also reactive with RS1G25. Two clones (2.1 and 3B.230) reacted with antigen prepared from RS1G25 but not RS1G31, indicating specificity for an antigen encoded by HSV-2 between approximately 0.59 and 0.67 map units.

gC-specific CD4+ T-cell clone. As the gene for gC2 is located at approximately 0.64 map units (41), the two clones reactive with RS1G25 but not RS1G31 (2.1 and 3B.230) were evaluated for proliferative responses to affinity-purified gC2 and special HSV strains (Table 4). Clone 3B.230 had a specific proliferative response to gC2. This clone also proliferated in response to the recombinant HSV-1 strain MP801-1, expressing the HSV-2 gC gene, and failed to proliferate in response to the HSV-2 strain G_PS1, which does not express gC2. Subclones of 3B.230 obtained at one cell per well displayed an identical pattern of reactivity (data not shown). Clone 3B.230 was also reactive in cytotoxicity assays with RS1G25 and MP801-1 but not RS1G31 or G_PS1 (Fig. 2), the same pattern of reactivity obtained in proliferation assays. In contrast, clone 2.1 did not proliferate in response to MP801-1 or purified gC2 and did react with the G_PS1, indicating reactivity with a HSV-2 type-specific epitope not contained within gC2.

CD4+ T-cell clones with specificity for VP16 and other antigens mapping to RS1G31. Eight lesion-derived CD4+ T-cell clones, including at least one from each patient, reacted with an HSV-2 epitope contained within IRV RS1G31 and thus mapping to approximately 0.67 to 0.73 map units (Table 3). One of these, clone 1A.B.25.1, proliferated in response to the IRV RP-2 (Table 5), which contains only the HSV-2 gene

TABLE 4. Proliferative response of lesion-derived CD4+ clones 2.1 and 3B.230 to purified gC2 and antigens prepared from various HSV strains

Clone	Stimulation index						
	HSV-1	HSV-2	RS1G25	RS1G31	MP801-1[a]	G_PS1[b]	gC2[c]
2.1	1.8	**5.7**	**5.5**	1.1	0.8	**6.1**	1.4
3B.230	0.8	**23**	**22**	1.4	**35**	1.0	**48**

[a] HSV-1 strain containing the gene for gC of HSV-2.
[b] HSV-2 strain which does not express gC.
[c] Used at 2 μg/ml (final concentration).

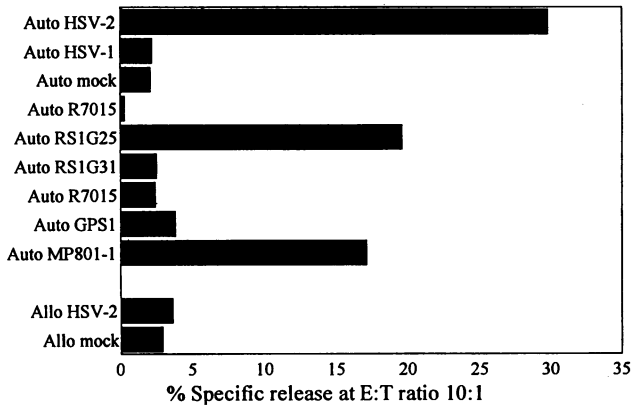

FIG. 2. Cytotoxic activity of gC2-specific CD4+ T-cell clone 3B.230 against infected and uninfected autologous (Auto) and allogeneic (Allo) LCL. HSV-1 × HSV-2 IRV RS1G25 contains HSV-2 DNA from approximately 0.59 to 0.73 map units within an HSV-1 background. MP801-1 is an HSV-1 strain containing the gene for gC of HSV-2. G_PS1 is an HSV-2 strain which does not express gC. Allogeneic LCL were mismatched at HLA class I and II loci. Data are percent specific release at an effector-to-target (E:T) ratio of 10:1.

for VP16 within an HSV-1 background. Clone 1A.B.25.1 is a subclone of 1A.B.25 (Table 3). Clones 2.3, 3B.294, 4.1A11, 4.2E1, and 4.2F10 did not react with RP-2 (Table 5). Clones 4.3C3 and 5.5 were not available for evaluation with RP-2 antigen.

Allogeneic PBMC from patient 5, sharing HLA DR3 and DQ2 with patient 1, presented HSV-2 antigen to clone 1A.B.25.1 (Table 6). Alloreactivity against the patient 5 PBMC was not present, as incorporation of [^{3}H]thymidine in response to these allogeneic PBMC and mock antigen was less than 500 cpm (data not shown). Proliferation of clone 1A.B.25.1 to autologous PBMC and HSV-2 antigen was inhibited more than 90% by anti-HLA DQ MAb SPV-L3 but not inhibited by anti-HLA DR or anti-HLA DP MAbs (Table 6), identifying the restriction element as DQ2. A fusion protein bearing the activation domain of VP16 of HSV-1 or HSV-2 linked to the DNA-binding domain of the yeast GAL4 protein was purified after expression in *E. coli*. Dose-dependent proliferative responses were observed at HSV-2 fusion protein concentrations of between 0.5 and 8 μg/ml (Fig. 3). No proliferation was elicited by the HSV-1 fusion protein. Peptides containing amino acids 425 to 439 (LRLDGEEVDMTPADA) and 430 to 444 (EEVDMTPADALDDFD) of VP16 of HSV-2 (11) both induced specific proliferative responses (Fig. 3).

TABLE 5. Proliferative responses of type-specific lesion-derived CD4+ clones recognizing an HSV-2 antigen mapping to RS1G31 (approximately 0.67 to 0.73 map units) to RP-2 antigen

Clone	Stimulation index[a]			
	HSV-1	HSV-2	RS1G31	RP-2
1A.B.25.1	3.0	52	85	**52**
2.3	1.1	30	46	1.6
3B.294	1.8	21	37	3.2
4.1A11	0.9	21	28	2.1
4.2E1	1.3	26	29	2.7
4.2F10	3.5	69	77	4.6

[a] Antigens used at 1:100 (final dilution) except for clones 4.1A11 and 4.2E1 (1:30 [final dilution]).

TABLE 6. Lesion-derived CD4+ T-cell clone 1A.B.25 is restricted by HLA DQ2

APC[a]	MAb	Stimulation index
Autologous	None	22
	Anti-DR	27
	Anti-DP	27
	Anti-DQ	1.5
Allogeneic	None	34

[a] Autologous PBMC are HLA DR3,11, DQ2, 7; allogeneic PBMC are HLA DR3, DQ2.

DISCUSSION

Limited information concerning the antigenic specificity of human HSV-specific T lymphocytes is available. Secondary in vitro restimulation of PBMC with HSV antigen prior to cloning has previously been necessary to obtain CD4+ HSV-specific T-cell clones (52). It is not known to what extent the antigenic specificities and effector functions of clones obtained with this method reflect the properties of T lymphocytes that have migrated to, and been activated within, recurrent HSV lesions. CD4+ T-cell clones obtained in this manner have been primarily specific for gB and gD (52). We have used the in situ enrichment of HSV-specific T cells in recurrent HSV-2 lesions (23) to obtain T cells for cloning without the requirement for in vitro restimulation with HSV antigen.

The T-cell cultures used in this study were believed to be clonal. It was necessary to initially plate the lesion cells at greater than one cell per well because of the low purity of lymphocytes (<1%) in the lesion cell preparations. Since the maximum percentage of wells positive for growth was 48.7% (23), the average number of lymphocytes plated per well is estimated to be less than one (20, 23). Since approximately 10% of all lesion-derived T-cell cultures were HSV specific (23), it is very unlikely that more than one HSV-specific T-cell clone would be represented within an individual culture of lesion lymphocytes. All of the cultures tested were >97% CD4+ and <3% CD8+. Multiple subclones were evaluated for

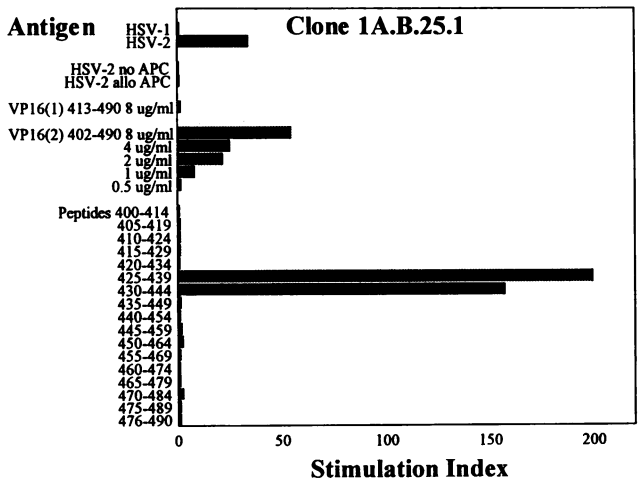

FIG. 3. Proliferative responses of lesion CD4+ clone 1A.B.25.1 to HSV antigens, purified recombinant GAL4-VP16 fusion proteins containing amino acids 1 to 147 of yeast GAL4 linked to amino acids 413 to 490 of VP16 (HSV-1) or amino acids 402 to 490 of VP16 (HSV-2), and peptides from VP16 of HSV-2. Allogeneic (allo) PBMC were mismatched at HLA class I and II loci.

clones 1A.B.25, 2.3, 3B.134, 3B.230, 3B.268, 4.2E1, and 4.2F10, and all were found to retain specific proliferative responses to HSV-2 antigen (23). The antigenic specificity of subclones, as determined by IRV mapping or reactivity with HSV glycoproteins, has been identical with that of the parent culture in all of 12 clones tested (data not shown). In addition, no cases of reactivity of HSV-2 type-specific T-cell clones with more than one nonoverlapping IRV were noted (Table 3).

The lesion $CD4^+$ response appears to be directed against a variety of viral epitopes. Using lesion-derived HSV-specific $CD4^+$ T-cell clones from five individuals, we identified at least 10 different epitopes: 1 to 3, epitopes contained within gB, gC, and gD; 4, a type-specific HSV-2 epitope contained within amino acids 425 to 444 of VP16; 5, a type-specific HSV-2 epitope or epitopes mapping to approximately 0.30 to 0.46 map units; 6, a type-specific HSV-2 epitope or epitopes mapping to approximately 0.59 to 0.67 map units but not gC; 7, a type-specific HSV-2 epitope (or epitopes) mapping to approximately 0.67 to 0.73 map units but not VP16; 8, a type-specific HSV-2 epitope (or epitopes) mapping to approximately 0.82 to 1.0 map units; 9, a type-specific HSV-2 epitope (or epitopes) not mappable with the current panel of IRV; and 10, a type-common epitope (or epitopes) in addition to those present in recombinant soluble gB and gD.

It is of interest that each of the five patients studied yielded at least one lesion-derived HSV-2 type-specific $CD4^+$ T-cell clone reactive with a HSV-2 epitope present within IRV RS1G31 and thus mapping to the approximate region of 0.67 to 0.73 map units. The maximum left-hand border of HSV-2 sequences contained within IRV RS1G31 is defined by the presence of the HSV-1 *Kpn*I-A to -Y′ cleavage site at nucleotide 103101 (31), within the HSV-1 UL47 coding sequence (26). The minimum left-hand border of HSV-2 sequences in RS1G31 has been mapped in the present experiments to be within or 5′ of the DNA encoding amino acids 425 to 444 of VP16 (UL48) of HSV-2. The minimum right border of the HSV-2 sequences within RS1G31 is defined by the presence of the HSV-2 *Eco*RI-L to -H cleavage site (31); this site cannot be precisely placed with published sequence information. The maximal right-hand border of HSV-2 sequences within IRV RS1G31 is defined by the absence of the HSV-2 *Bgl*II-I to -H cleavage site (31). The crossover point must lie to the left of the 5′ end of the HSV-2 UL53 gene, since the HSV-2 *Bgl*II-I to -H cleavage site is not present in the UL53 coding sequence (15) or the remaining rightward region of UL of HSV-2 (25, 34). IRV RS1G31 may, therefore, contain HSV-2 coding sequences for some of UL47 of HSV-2, but the HSV-2 sequences do not extend into the coding region of UL53 of HSV-2.

Of the total of eight HSV-2 type-specific clones mapping to IRV RS1G31, only one of six available for testing reacted with IRV RP-2 and was thus specific for the UL48 gene product VP16. The 0.67–0.73 region of the HSV-2 genome contains, in addition to VP16, the genes for the abundant tegument proteins UL47 and UL49 and the genes for the probable membrane glycoprotein UL49.5 or UL49A (4, 5, 16, 54). The UL50 and UL52 protein products are perhaps less likely candidates for $CD4^+$ T-cell antigens, since they are present in small amounts in infected cells (30, 47) and have not been identified in virions. Little is known about the UL51 gene product of HSV (3). Study of additional HSV-2 lesions and finer definition of the T-cell epitope(s) will allow exploration of the possibility that an immunodominant HSV-2 type-specific $CD4^+$ antigen may be encoded by a gene in this portion of the genome.

The lesion-derived clone 1A.B.25 is the first published $CD4^+$ T-cell clone reactive with VP16. VP16 is an abundant virion tegument protein, present as 400 to 2,000 molecules per virion (6, 29). Our results are consistent with the hypothesis that preformed virion proteins, rather than endogenously synthesized viral proteins, would be expected to recognized by the $CD4^+$ T-cell response (7). The predicted amino acid sequences of VP16 of HSV-1 and HSV-2 are 86% identical, with maximal divergence in the carboxy-terminal activation subdomain (11). Type-specific T-cell clone 1A.B.25 is restricted by HLA DQ2, as determined from data from partially HLA matched allogeneic APC and inhibition of proliferative responses to autologous APC by various anti-HLA class II MAbs (Table 6). Inhibition of the proliferative response to allogeneic, HLA DQ2-bearing APC plus antigen by anti-HLA DQ MAb would also be expected; this confirmatory experiment and identification of further HLA restriction elements of additional T-cell clones are under way. The naturally processed epitope of VP16 of HSV-2 most likely includes the overlap region, amino acids 430 to 439, shared by the two antigenic peptides 425 to 439 and 430 to 444 (Fig. 3). This overlap region (EEVDMTPADA) shares negatively charged amino acids at positions 1 and 9 and alanine at position 8 with positions 2, 9, and 10 of the HLA DQ2-restricted peptide IDVWLGGLAE NSLP (human thyroid peroxidase positions 632 to 645 [14, 21]).

A low proportion (5 of 47 [11%]) of lesion-derived $CD4^+$ T-cell clones were specific for the abundant membrane glycoprotein gB, gC, or gD. Several factors may have limited detection of T-cell clones with these specificities. First, the recombinant gB2 and gD2 molecules used in this study have C-terminal deletions; the reagents include 79 and 82%, respectively, of the predicted amino acid sequences of the native proteins after cleavage of N-terminal signal sequences (44, 55). T-cell clones reactive with whole HSV antigens would be scored as negative for gB2 or gD2 if they were specific for epitopes not included in the recombinant soluble glycoproteins. Second, only HSV-2 type-specific T-cell clones reacting with RS1G25 but not RS1G31 antigens were screened for gC2 reactivity; type-common, gC-specific T-cell clones would be missed by this method. However, the 31% divergence between the amino acid sequences of gC1 and gC2 (41) makes it likely that most T-cell epitopes of HSV gC will be type specific. Third, the amount of glycoprotein antigen used in proliferation assays may have been suboptimal for some T-cell clones, although a 2-μg/ml concentration of HSV gB, gC, and gD has previously been shown to be optimal for eliciting proliferative responses from bulk PBMC and PBMC-derived $CD4^+$ T-cell clones (43, 52). Even allowing for these potential limiting factors, however, there is still a marked contrast between the previously reported finding that most PBMC-derived HSV-specific $CD4^+$ T-cell clones are specific for gB or gD (52) and the results of the present study with lesion-derived T-cell clones.

The large size of the HSV genome (33) and the diverse peptides bound to various human HLA class II molecules (9) lead to the prediction that a large number of human $CD4^+$ epitopes are present with HSV-2. Considerable diversity was detected among the antigens recognized by $CD4^+$ T-cell clones recovered from individual patients. For example, a minimum of six different specificities were detected for patient 5: gB2, at least one HSV-2 type-specific epitope encoded by HSV-2 DNA present within each of the three IRV (RH1G7, RS1G31, and R7015), at least one type-common epitope in addition to gB2, and at least one HSV-2 type-specific epitope encoded by HSV-2 gene sequence(s) not contained within the panel of IRV. The overall detection of at least 10 T-cell epitopes

recognized by lesion-infiltrating HSV-specific CD4+ T cells provides a minimum estimate of the diversity of viral epitopes because of the small number of patients studied, the limited availability of purified viral proteins, and, for HSV-2 type-specific T-cell clones, the incomplete coverage and crude mapping of the HSV-2 genome provided by the current panel of IRV. We plan to combine epitope mapping with additional IRV and determination of individual clonal HLA restriction elements with consensus motifs for HLA class II-binding peptides (9) and HSV sequence data to define epitopes at the peptide level.

The biological roles of lesion-infiltrating antigen-specific CD4+ T cells in the resolution of recurrent HSV-2 lesions are not known. Patients with deficiencies of CD4+ cell function, such as advanced human immunodeficiency virus infection, have delayed and incomplete resolution of recurrent HSV-2 infection (46). In addition to the gC- and gD-specific clones described above, only one additional HLA-restricted, HSV-specific CD4+ T-cell clone with CTL activity was detected among the 47 T-cell clones described in this study (3/47 = 6%). In contrast, 30 to 80%, depending on the donor, of PBMC-derived HSV-specific CD4+ T-cell clones obtained with secondary in vitro stimulation have had CTL activity (52). The apparent difference may be related to culture conditions, as progressive increases in apparent CTL activity with in vitro restimulation with antigen have been described for human CD4+ clones specific for other viruses (17). Additional potential effector functions include secretion of lymphokines with antiviral or immunomodulatory activity, including gamma interferon (18, 19), and B-cell helper activity (49). Studies of lymphokines produced in situ in HSV lesions and in vitro by HSV-specific CD4+ T cells are under way.

In summary, CD4+ T cells present within human recurrent HSV-2 lesions recognize a diverse set of type-common and HSV-2 type-specific antigens. T-cell clones specific for gB and gD have been detected, extending observations with PBMC-derived T-lymphocyte clones. Human T-cell clones specific for gC and the virion tegument protein VP16 have been detected for the first time, and at least five additional HSV-2 type-specific antigens have been defined by using patterns of reactivity with HSV-1 × HSV-2 IRV. In one patient for which two recurrent HSV-2 lesions have been studied, a T-cell clone reactive with a HSV-2 type-specific antigen mapping to the same region of the HSV-2 genome (approximately 0.30 to 0.46 map units) was recovered from both lesions. Study of additional lesions and genetic analysis of the T-cell receptor genes of T-cell clones derived from sequential HSV lesions may determine whether clonotypic T cells participate in the host reaction to serial HSV recurrences within an individual patient. Continued work on the diversity and antigenic specificity of the human CD4+ response to HSV may further our understanding of the processes by which the host controls human recurrent herpes simplex infection.

ACKNOWLEDGMENTS

Specimens were collected by Gail Barnum, Michael Remington, Peter Tretheway, and Mary Shaughnessy. Invaluable technical assistance was given by Annette Peck and Hiyam Abbo. HLA typing was performed by Brenda Nisperos and John Hansen at the Fred Hutchinson Cancer Research Center, Seattle, Wash. Bernard Roizman and Patricia Spear, University of Chicago, provided recombinant HSV strains. David B. Lewis and Tom Cotner, University of Washington, Seattle, provided MAbs and helpful advice.

This work was supported by National Institutes of Health grants AI31448 and AI34616 (D.M.K.), AI20381 (L.C.), and AI27323 (S.J.T.) and by a Junior Faculty Research Award from the American Cancer Society (S.J.T.).

REFERENCES

1. **Ashley, R., C. Critchlow, M. Shurtleff, and L. Corey.** 1988. Differential effect of systemic acyclovir treatment of genital HSV-2 infections on antibody responses to individual HSV-2 proteins. J. Med. Virol. **24**:309–320.
2. **Ashley, R. A., J. Militoni, F. Lee, A. Nahmias, and L. Corey.** 1988. Comparison of Western blot (immunoblot) and glycoprotein G-specific immunoblot for detecting antibodies to herpes simplex types 1 and 2 in human sera. J. Clin. Microbiol. **26**:662–667.
3. **Barker, D. E., and B. Roizman.** 1990. Identification of three genes nonessential for growth in cell culture near the right terminus of the unique sequences of long component of herpes simplex virus type 1. Virology **177**:684–691.
4. **Barker, D. E., and B. Roizman.** 1992. The unique sequence of the herpes simplex virus 1 L component contains an additional translated open reading frame designated UL49.5. Virology **66:** 562–566.
5. **Barnett, B. C., A. Dolan, E. A. R. Telford, A. J. Davison, and D. J. McGeoch.** 1992. A novel herpes simplex virus gene (UL49A) encodes a putative membrane protein with counterparts in other herpesviruses. J. Gen. Virol. **73**:2167–2171.
6. **Batterson, W., and B. Roizman.** 1983. Characterization of the herpes simplex virus-associated factor responsible for the induction of α genes. J. Virol. **46**:371–377.
7. **Brodsky, F. M., and L. E. Guagliardi.** 1991. The cell biology and antigen processing and presentation. Annu. Rev. Immunol. **9**:707–744.
8. **Burke, R. L.** 1991. Development of a herpes simplex virus subunit glycoprotein vaccine for prophylactic and therapeutic use. Rev. Infect. Dis. **13**:S906–S911.
9. **Chicz, R. M., R. G. Urban, J. C. Gorga, D. A. A. Vignali, W. S. Lane, and J. L. Strominger.** 1993. Specificity and promiscuity among naturally processed peptides bound to HLA-DR alleles. J. Exp. Med. **178**:27–47.
10. **Conley, A. J., D. M. Knipe, P. C. Jones, and B. Roizman.** 1981. Molecular genetics of herpes simplex virus. VII. Characterization of a temperature-sensitive mutant produced by in vitro mutagenesis and defective in DNA synthesis and accumulation of τ polypeptides. J. Virol. **37**:191–206.
11. **Cress, A., and S. J. Triezenberg.** 1991. Nucleotide and deduced amino acid sequence of the gene encoding virion protein 16 of herpes simplex virus type 2. Gene **103**:235–238.
12. **Cunningham, A. L., and J. R. Noble.** 1989. Role of keratinocytes in human recurrent herpetic lesions. J. Clin. Invest. **83**:490–496.
13. **Cunningham, A. L., R. R. Turner, A. C. Miller, M. F. Para, and T. C. Merigan.** 1985. Evolution of recurrent herpes simplex lesions: an immunohistologic study. J. Clin. Invest. **75**:226–233.
14. **Dayan, C. M., M. Londei, A. E. Corcoran, et al.** 1991. Autoantigen recognition by thyroid-infiltrating T cells in Graves disease. Proc. Natl. Acad. Sci. USA **88**:7415–7419.
15. **Debroy, C.** 1990. Nucleotide sequence of the herpes simplex type 2 syn gene that causes cell fusion. Gene **88**:275–277.
16. **Elliott, G. D., and D. M. Meredith.** 1992. The herpes simplex virus type 1 tegument protein VP22 is encoded by gene UL49. J. Gen. Virol. **73**:723–736.
17. **Fleischer, B.** 1984. Acquisition of specific cytotoxic activity by human T4+ lymphocytes in culture. Nature (London) **308**:365–367.
18. **Hendricks, R. L., T. M. Tumpey, and A. Finnegan.** 1992. IFN-τ and IL-2 are protective in the skin but pathologic in the corneas of HSV-1-infected mice. J. Immunol. **149**:3023–3028.
19. **Ho, M.** 1990. Interferon as an agent against herpes simplex virus. J. Invest. Dermatol. **95**:158S–160S.
20. **Hudson, L., and F. C. Hay.** 1989. Practical immunology, 3rd ed. Blackwell Scientific Publications, Oxford.
21. **Kimura, S., T. Kotani, O. W. McBride, et al.** 1987. Human thyroid peroxidase: complete cDNA and protein sequence, chromosome mapping, and identification of two alternatively spliced mRNAs. Proc. Natl. Acad. Sci. USA **84**:5555–5559.
22. **Kit, S., M. Kit, H. Qavi, D. Trkula, and H. Otsuka.** 1983. Nucleotide sequence of the herpes simplex virus type 2 (HSV-2) thymidine kinase gene and predicted amino acid sequence of thymidine kinase polypeptide and its comparison with the HSV-1

thymidine kinase gene. Biochim. Biophys. Acta **741**:158.
23. **Koelle, D. M., H. Abbo, A. Peck, K. Ziegweid, and L. Corey.** Direct recovery of HSV-specific T lymphocyte clones from human recurrent HSV-2 lesions. J. Infect. Dis., in press.
24. **Leung, K. N., A. A. Nash, D. Y. Sia, and P. Wildy.** 1984. Clonal analysis of T-cell responses to herpes simplex virus: isolation, characterization and antiviral properties of an antigen-specific helper T-cell clone. Immunology **53**:623–633.
25. **McGeoch, D. J., C. Cunningham, G. McIntyre, and A. Dolan.** 1991. Comparative sequence analysis of the long repeat regions and adjoining parts of the long unique regions in the genomes of herpes simplex viruses types 1 and 2. J. Virol. **72**:3057–3075.
26. **McGeoch, D. J., M. A. Dalrymple, A. J. Davison, et al.** 1988. The complete DNA sequence of the long unique region of herpes simplex virus type 1. J. Gen. Virol. **69**:1531–1574.
27. **Moretta, A., G. Pantaleo, L. Moretta, J.-C. Cerottini, and M. C. Mingari.** 1983. Direct demonstration of the clonogenic potential of every human peripheral blood T cell. J. Exp. Med. **157**:743–754.
28. **Nash, A. A., A. Jayasuriya, J. Phelan, S. P. Cobbold, H. Waldman, and T. Prospero.** 1987. Different roles for the L3T4+ and Lyt2+ T cell subsets in the control of an acute herpes simplex virus infection of the skin and nervous system. J. Gen. Virol. **68**:825–833.
29. **O'Hare, P.** 1992. Targets for antiviral chemotherapy: herpes simplex virus regulatory protein, Vmw65. Antiviral Chem. Chemother. **2**:1–7.
30. **Olivo, P. D., N. J. Nelson, and M. D. Challberg.** 1989. Herpes simplex virus type 1 gene products required for DNA replication: identification and overexpression. J. Virol. **63**:196–204.
31. **Para, M. F., K. M. Zezulak, A. J. Conley, M. Weinberger, K. Snitzer, and P. G. Spear.** 1983. Use of monoclonal antibodies against two 75,000-molecular-weight glycoproteins specified by herpes simplex virus type 2 in glycoprotein identification and gene mapping. J. Virol. **45**:1223–1227.
32. **Purves, F. C., D. Spector, and B. Roizman.** 1991. The herpes simplex virus type 1 protein kinase encoded by the US3 gene mediates posttranslational modification of the phosphoprotein encoded by the UL34 gene. J. Virol. **65**:5757–5764.
33. **Roizman, B., and A. E. Sears.** 1990. Herpes simplex viruses and their replication. p. 1795–1841. *In* B. N. Fields and D. M. Knipe (ed.), Virology, 2nd ed. vol. 2. Raven Press, Ltd., New York.
34. **Roizman, B., and M. Tognon.** 1983. Restriction endonuclease patterns of herpes simplex virus DNA: application to diagnosis and molecular epidemiology. Curr. Top. Microbiol. Immunol. **104**:274–286.
35. **Royston, I., M. B. Omary, and I. S. Trowbridge.** 1981. Monoclonal antibodies to a human T-cell antigen and Ia-like antigen in the characterization of lymphoid leukemia. Transplant. Proc. **13**:761–766.
36. **Sadowski, I., J. Ma, S. Triezenberg, and M. Ptashne.** 1988. Gal4-VP16 is an unusually potent transcriptional activator. Nature (London) **335**:563–564.
37. **Schmidt, N. J.** 1989. Cell culture procedures for diagnostic virology, p. 57–100. *In* N. J. Schmidt and R. W. Emmons (ed.), Diagnostic procedures for viral, rickettsial and chlamydial infections, 6th ed. American Public Health Association, Washington, D.C.

37a. **Shen, F., J. G. Reddy, R. Pichyangkura, and S. Triezenberg.** Unpublished data.

38. **Smith, L. M., H. R. Petty, P. Parham, and H. M. McConnell.** 1982. Cell surface properties of HLA antigens in Epstein-Barr virus-transformed cell lines. Proc. Natl. Acad. Sci. USA **79**:608–612.
39. **Spits, H., J. Borst, M. Giphart, J. Cougan, C. Terhorst, and T. E. De Vries.** 1984. HLA-DC antigens can serve as recognition elements for human cytotoxic T lymphocytes. Eur. J. Immunol. **14**:299–304.
40. **Spruance, S. L., and F. S. Chow.** 1980. Pathogenesis of herpes simplex virus in cultures of epidermal cells from subjects with frequent recurrences. J. Infect. Dis. **142**:671–675.
41. **Swain, M. A., R. W. Peet, and D. A. Galloway.** 1985. Characterization of the gene encoding herpes simplex virus type 2 glycoprotein C and comparison with the type 1 counterpart. J. Virol. **53**:561–569.
42. **Tigges, M. A., D. M. Koelle, K. Hartog, R. E. Sekulovich, L. Corey, and R. L. Burke.** 1992. Human $CD8^+$ herpes simplex virus-specific cytotoxic T lymphocyte clones recognize diverse virion protein antigens. J. Virol. **66**:1622–1634.
43. **Torseth, J. W., G. H. Cohen, R. J. Eisenberg, P. W. Berman, L. A. Lasky, C. P. Cerini, C. J. Heilman, S. Kerwar, and T. C. Merigan.** 1987. Native and recombinant herpes simplex virus type 1 envelope proteins induce human immune T-lymphocyte responses. J. Virol. **61**:1532–1539.
44. **Watson, R. J.** 1983. DNA sequence of the herpes simplex virus type 2 glycoprotein D gene. Gene **26**:307–312.
45. **Weinheimer, S. P., B. B. Boyd, S. K. Durham, J. L. Resnick, and D. R. O'Boyle.** 1992. Deletion of the VP16 open reading frame of herpes simplex virus type 1. J. Virol. **66**:258–269.
46. **Whitley, R. J.** 1990. Herpes simplex viruses, p. 1843–1887. *In* B. N. Fields and D. M. Knipe (ed.), Virology, 2nd ed. Raven Press, Ltd., New York.
47. **Williams, M. V.** 1988. Herpes simplex virus-induced dUTPase: target site for antiviral chemotherapy. Virology **166**:262–264.
48. **Yasukawa, M., A. Inatsuki, T. Horiuchi, and Y. Kobayashi.** 1991. Functional heterogeneity among herpes simplex virus-specific human CD4+ T cells. J. Immunol. **146**:1341–1347.
49. **Yasukawa, M., A. Inatsuki, and Y. Kobayashi.** 1988. Helper activity in antigen-specific antibody production mediated by CD4+ human cytotoxic T cell clones directed against herpes simplex virus. J. Immunol. **140**:3419–3425.
50. **Yasukawa, M., and Y. Kobayashi.** 1985. Inhibition of herpes simplex virus replication in vitro by human cytotoxic T cell clones and natural killer cell clones. J. Gen. Virol. **66**:2225–2229.
51. **Yasukawa, M., and J. M. Zarling.** 1984. Human cytotoxic T cell clones directed against herpes simplex virus-infected cells. I. Lysis restricted by HLA Class II MB and DR antigens. J. Immunol. **133**:422–427.
52. **Zarling, J. M., P. A. Moran, R. L. Burke, C. Pachl, P. W. Berman, and L. A. Lásky.** 1986. Human cytotoxic T cell clones directed against herpes simplex virus-infected cells. IV. Recognition and activation by cloned glycoproteins gB and gD-1. J. Immunol. **136**:4669–4673.
53. **Zezulak, K. M., and P. G. Spear.** 1984. Mapping of the structural gene for the herpes simplex virus type 2 counterpart of herpes simplex virus type 1 glycoprotein C and identification of a type 2 mutant which does not express this glycoprotein. J. Virol. **49**:741–747.
54. **Zhang, Y., and J. L. C. McKnight.** 1993. Herpes simplex virus type 1 UL46 and UL47 deletion mutants lack VP11 and VP12 or VP13 and VP14, respectively, and exhibit altered thymidine kinase expression. J. Virol. **67**:1482–1492.
55. **Zwaagstra, J. C., and W.-C. Leung.** 1987. The nucleotide sequence of herpes simplex virus type 2 (333) glycoprotein gB2 and analysis of predicted antigenic sites. Can. J. Microbiol. **33**:879–887.