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Mapping of a Herpes Simplex Virus Type 2-Encoded Function That Affects the Susceptibility of Herpes Simplex Virus-Infected Target Cells to Lysis by Herpes Simplex Virus-Specific Cytotoxic T Lymphocytes

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A function(s) involved in the altered susceptibility of herpes simplex virus type 2 (HSV-2)-infected cells to specific lysis by cytotoxic T lymphocytes was mapped in the S component of HSV-2 DNA by using HSV-1 × HSV-2 intertypic recombinants (RH1G44, RS1G25, R50BG10, A7D, and C4D) and HSV-1 MP. Target cells infected with R50BG10, A7D, and C4D exhibited reduced levels of cytolysis, as did HSV-2-infected cells, whereas RH1G44 and RS1G25 recombinant-infected and HSV-1 MP-infected cells showed levels of lysis equal to that of HSV-1 KOS-infected cells. The intertypic recombinants R50BG10, RS1G25, RH1G44, and HSV-1 MP induced cross-reactive cytotoxic T lymphocytes. Coinfection of cells with HSV-1 KOS and either HSV-2 186 or R50BG10 recombinant also resulted in a decrease in the level of specific lysis by anti-HSV cytotoxic T lymphocytes.

Previous studies from our laboratory (6) and others (11, 13) have focused on the involvement of herpes simplex virus (HSV)-specific glycoproteins in the T cell-mediated lysis of HSV-infected cells. In comparing the susceptibility of HSV type 1 (HSV-1) and HSV type 2 (HSV-2)-infected cells to specific lysis cytotoxic T lymphocytes (CTLs) generated either to HSV-1 or HSV-2, it was discovered that HSV-2-infected cells were significantly less susceptible to lysis by CTLs (5). This lowered susceptibility of HSV-2-infected cells to specific CTLs was neither related to the replication of HSV-2 in mouse (*H-2*^{b}) cells nor due to the lack of expression of HSV-2 glycoproteins on the surface of HSV-2-infected target cells (5). In addition, HSV-2-infected cells were as efficient in blocking the activity of HSV-specific CTLs as were HSV-1-infected cells, although the degree of blocking achieved with both infected cell types was low (5). As a first approach to determine the reasons behind this phenomenon, the possibility was considered that the HSV-2 glycoproteins recognized as target antigens by anti-HSV CTLs are involved in the alteration of susceptibility to CTL-mediated lysis of HSV-2-infected cells. The HSV-2 genome specifies five glycoproteins. Glycoproteins gA/B (2, 3, 16, 30) and gC, which was previously designated gF (2, 3, 20), have been mapped to the L component of the genome. Glycoproteins gD (16, 30), gE (2, 3, 19), and another glycoprotein of molecular weight 92,000 to 120,000 (92K to 120K) (15, 16) map in the S component of HSV-2 genome. The 92 to 120K glycoprotein has been designated gG (B. Roizman, personal communication). HSV-1 genome specifies four glycoproteins: gA/B, gC, gD, and gE (4, 18, 32). Glycoproteins gA (32) has been reported to be antigenically related to gB (14), and the two glycoprotein species have not been differentiated serologically either by polypeptide-specific antisera (8–10) or by monoclonal antibodies (21). The HSV glycoproteins have been implicated in the immunoregulation of herpetic infections involving both humoral and cell-mediated mechanisms (1, 5, 7, 13, 23, 24, 28).

To resolve the mechanism of lowered susceptibility of HSV-2-infected target cells to CTL-mediated lysis, we made use of HSV-1 × HSV-2 intertypic recombinants which would allow us to identify regions on the HSV-2 genome which may be directly involved in the lowered susceptibility of HSV-2-infected cells. The results of these studies demonstrate that the function(s) determining the lowered susceptibility of HSV-2-infected cells maps in the S component of the HSV-2 genome, and the function(s) appears to be dominant based on the reduced cytolysis of cells coinfected with HSV-1 and HSV-2.

MATERIALS AND METHODS

Cells and cell culture. Monolayer cultures of the human cell line HEp-2 were used for the preparation of virus stocks. A continuous line of African green monkey kidney cells (Vero) was used for virus plaque assays. Both Hep-2 and Vero cells were grown at 37°C in Dulbecco modified Eagle medium supplemented with 10% fetal bovine serum and containing 0.075 or 0.225% $NaHCO_3$ for cultures in closed or open vessels, respectively. C57BL/6 mouse embryo fibroblasts transformed by simian virus 40 (B6/WT-3) (26) were used as target cells in the ^{51}Cr release assay.

Viruses and virus assays. The HSV-1 strain KOS and HSV-2 strain 186 were kindly provided by Priscilla A. Schaffer, and HSV-1 MP and HSV-1 × HSV-2 recombinants R50BG10, RH1G44, RS1G25, A7D, and C4D were kindly provided by Bernard Roizman. The origin of recombinants and the parental strains used to generate these recombinants are shown in Table 1. Virus stocks were prepared in HEp-2 cells and titrated in Vero cells by a plaque assay utilizing a 2% methylcellulose overlay (31). All virus stocks were stored at −70°C.

Generation of CTLs. CTLs capable of specifically killing syngeneic HSV-infected cells were generated by immunizing C57BL/6 (*H-2*^{b}) mice (Jackson Laboratories, Bar Harbor, Maine) with 10^5 PFU of virus in each hind footpad. The

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TABLE 1. HSV strains and intertypic recombinant viruses used

Parental HSV strains	HSV strain or recombinant virus	Approximate location of HSV-2 DNA in recombinants (map units)	Reference
	HSV-1 (KOS)		28, 29
	HSV-1 (MP)		32
	HSV-2 (186)		12
HSV-1 (mP)*tsHA*1 × HSV-2 (G)	RH1G44	0.35–0.43	8
HSV-1 (mP)*tsSB*1 × HSV-2 (G)	RS1G25	0.58–0.72	20; A. J. Conley and B. Roizman (unpublished)
HSV-1 (mP)*ts*50B × HSV-2 (G)	R50BG10	0.82–1.0	33
HSV-1(17)*tsJ*(PAA^r) × HSV-2 (GP6)	A7D	0.78–1.0	17, 30
HSV-1 (HFEM)*tsN*102(PAA^r) × HSV-2 (186)	C4D	0.0–0.28, 0.72–1.0	17, 30

draining lymph nodes were excised 5 days post-immunization, and lymphocyte suspensions were prepared by gently pressing the lymph nodes through a 60-gauge stainless steel wire mesh. Viable cells were counted by trypan blue exclusion and suspended at 4×10^6 lymphocytes per ml in RPMI 1640 medium containing 2×10^{-5} M 2-mercaptoethanol, 20 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) buffer, 100 U of penicillin per ml, 100 μg of streptomycin per ml, 0.03% glutamine, 0.225% $NaHCO_3$, and 10% heat-inactivated (56°C, 30 min) fetal bovine serum. Lymphocytes (2×10^7) were added to 60-mm tissue culture dishes and incubated at 37°C in 5% CO_2 for 3 days. For controls, lymphocytes from nonimmunized mice were similarly prepared and cultured. The procedure has been described previously (5, 6).

^{51}Cr release assay. The ^{51}Cr release assay was performed as described previously (6). Briefly, confluent monolayers of target cells growing in tissue culture flasks (75 cm^2) were infected with the appropriate HSV strain or recombinant at a multiplicity of infection (MOI) of 2.5, unless stated otherwise, and 200 μCi of ^{51}Cr (specific activity, 200 Ci/g; New England Nuclear Corp., Boston, Mass.) was added to each flask. The cells were placed at 37°C for 14 to 16 h. Then 2×10^4 cells in 0.1 ml were added to glass culture tubes (10 by 75 mm) with an equal volume of effector lymphocytes, at an effector-to-target cell ratio of 40:1 unless otherwise stated, and incubated at 37°C for 5 h.

RESULTS

Susceptibility of HSV-1 × HSV-2 intertypic recombinant-infected B6/WT-3 cells to lysis by anti-HSV CTLs. CTLs generated in C57BL/6 mice immunized with HSV-1 KOS or HSV-2 186 were used in a ^{51}Cr release assay to determine the susceptibility to lysis of target cells infected with HSV-1 × HSV-2 intertypic recombinant viruses. Recombinant viruses (RH1G44, RS1G25, and R50BG10) were isolated from cells cotransfected with HSV-1 DNA and restriction endonuclease digests of HSV-2 DNA (Table 1). Recombinants A7D and C4D were isolated by mixed infection of cells by HSV-1 and HSV-2 virions under selective pressure (Table 1). Recombinants express a full complement of HSV envelope glycoproteins, but each glycoprotein species is of only one parental type. Intertypic recombinants, therefore, provide a useful tool for determining the region of type 2 parental DNA responsible for susceptibility to lysis by CTLs. The target cells infected with HSV-1 MP, RH1G44, and RS1G25 were as efficiently lysed as the HSV-1 KOS-infected target cells, but the R50BG10-, A7D-, and C4D-infected target cells exhibited a lowered level of lysis, as did the HSV-2 186-infected target cells (Table 2). The data indicate that the function(s) that affects the HSV-2-infected target cell susceptibility is encoded within the HSV-2 sequences of R50BG10, A7D, and C4D, and therefore maps to the S component of the HSV-2 genome. We have previously shown (5) that the parental viruses used to generate the intertypic recombinants (Table 1) behave in a manner similar to that of the HSV-1 KOS and HSV-2 186 strains used in this study.

Induction of CTLs in C57BL/6 mice immunized with HSV-1 MP, RS1G25, RH1G44, and R50BG10. All HSV virus types examined generated CTLs in C57BL/6 mice (Table 3). The HSV-1 MP-infected target cells exhibited slightly higher values of specific lysis when tested in the ^{51}Cr release assay. It is of interest to note that HSV-1 MP causes polykaryocyte formation of the infected cell monolayer, and this may be involved in the increased percent ^{51}Cr release observed with HSV-1 MP-infected target cells. The data stress the importance of the right end of the HSV genome in the lowered susceptibility to lysis observed with HSV-2-infected and R50BG10-infected target cells.

Susceptibility of cells coinfected with HSV-1 KOS and intertypic recombinant R50BG10 to lysis by anti-HSV CTLs. Since HSV-2 and the recombinants R50BG10, A7D, and C4D exhibited a lowered level of lysis by anti-HSV CTLs, coinfection experiments were performed with R50BG10, HSV-2 186, and HSV-1 KOS. The B6/WT-3 target cells were infected with either HSV-1 KOS, HSV-2 186, or R50BG10, or were coinfected at a combined MOI of 5 with either HSV-1 KOS and HSV-2 186 or HSV-1 KOS and R50BG10. Cells infected with R50BG10 exhibited a susceptibility to lysis by anti-HSV CTLs similar to that of HSV-2 186-infected cells (Table 4). Coinfection of cells with HSV-1 KOS and HSV-2 186 resulted in low levels of ^{51}Cr release comparable to that of cells infected with HSV-2 186 alone. Coinfection of cells with HSV-1 KOS and R50BG10 also resulted in reduced levels of lysis by anti-HSV CTLs, as was observed with cells coinfected with HSV-1 KOS and HSV-2 186 and cells infected with HSV-2 186 alone. The data suggest that the HSV-2-encoded function(s) affecting the susceptibility of HSV-infected cells to lysis by HSV CTLs maps within the HSV-2 sequences of the HSV-1 × HSV-2 recombinant R50BG10, and further suggest the dominance of this HSV-2-coded function over HSV-1.

We were interested in determining whether the reduction of lysis of HSV-2-infected cells coinfected with HSV-2 could be overcome by increasing the MOI of HSV-1 KOS relative to the MOI of HSV-2 186. The B6/WT-3 target cells were infected with HSV-1 KOS at increasing MOI and with HSV-

TABLE 2. Susceptibility of HSV-1 × HSV-2 recombinant-infected target cells to lysis by anti-HSV CTLs

Target cells (B6/WT-3) infected by[a]:	Lymphocyte donor (C57BL/6) immunized with:	% Specific ^{51}Cr release[b]				
		Expt 1	Expt 2	Expt 3	Expt 4	Expt 5
HSV-1 KOS	HSV-1 KOS	42.3	79.1	43.1	37.8	41.6
	HSV-2 186	28.4	66.8	41.8	26.5	46.3
HSV-2 186	HSV-1 KOS	5.9	25.6	24.1	6.6	7.4
	HSV-2 186	9.0	24.2	26.9	5.5	13.9
HSV-1 MP	HSV-1 KOS			43.6		
	HSV-2 186			42.9		
RH1G44	HSV-1 KOS	62.4	65.1	39.0	32.9	
	HSV-2 186	42.6	49.6	38.2	24.2	
RS1G25	HSV-1 KOS			57.3		
	HSV-2 186			55.3		
R50BG10	HSV-1 KOS	10.2	26.9	30.1		
	HSV-2 186	6.9	26.6	30.7		
A7D	HSV-1 KOS				7.7	
	HSV-2 186				5.5	
C4D	HSV-1 KOS					15.0
	HSV-2 186					15.0
None	HSV-1 KOS	1.9	6.1	0.5	3.5	0
	HSV-2 186	4.3	5.3	3.4	3.7	0

[a] Target cells were infected with the designated virus strain at a MOI of 2.5 or mock infected, labeled with 200 μCi of ^{51}Cr, and harvested 14 h postinfection.

[b] Lymphocyte cytotoxicity was determined in a 5-h ^{51}Cr release assay at 37°C, at an effector-to-target cell ratio of 40:1 for experiments 1, 2, and 4, 50:1 for experiment 3, and 20:1 for experiment 5.

2 186 at a MOI of 1. Even when the MOI of HSV-1 KOS was 10-fold higher than that of HSV-2 186, the resultant increase in lysis of the coinfected target cells by anti-HSV CTLs was negligible (data not shown), indicating that the HSV-2 function(s) that affects the susceptibility to lysis by anti-HSV CTLs is dominant in cells coinfected with the type 1 virus and HSV-2 186.

Effect of superinfection with HSV-2 of HSV-1-infected cells on the susceptibility of target cells to CTL lysis. We attempted to determine the length of time of HSV-2 infection required for reducing the susceptibility of HSV-1-infected cells to CTL lysis. To accomplish this, B6/WT-3 cells were infected with HSV-1 and then with HSV-2 for 2, 4, 8, or 14 h, at which time the target cells were tested in a CTL assay. Infection of target cells with HSV-1 and HSV-2 for the entire time (14 h), or the superinfection of HSV-1-infected cells with HSV-2 for 8 h, resulted in the reduced lysis of target cells by anti-HSV CTLs (Table 5). On the other hand, superinfection of HSV-1-infected cells with HSV-2 for 2 or 4 h did not result in the lowered lysis of target cells by anti-HSV CTLs, suggesting that HSV-2 viral functions must be expressed to affect the susceptibility of HSV-1-infected cells to lysis by anti-HSV CTLs. It should be pointed out that evidence does exist that the HSV-1-infected cells can be superinfected with HSV-2 (27).

We considered the possibility that the reduced level of lysis of cells coinfected with HSV-1 and HSV-2 is due to the coinfection event itself. However, B6/WT-3 cells coinfected with two HSV-1 strains (KOS and 17) showed no reduction in lysis (data not shown). A further explanation may be that HSV-2 prevents the expression of HSV-1 glycoproteins after coinfection. However, cells coinfected with HSV-1 and HSV-2 were found to express glycoproteins of both types on the cell surface, as tested by the indirect immunofluorescence test (data not shown), using type-specific monoclonal antibody to HSV-1 gC (22) and to HSV-2 gC (2, 3), now tentatively designated gG (B. Roizman, personal communication), suggesting that the lowered susceptibility to CTLs of

TABLE 3. Induction of CTLs in C57BL/6 mice with MP and the HSV-1 × HSV-2 recombinants

Target cells (B6/WT-3) infected by[a]:	Lymphocyte donor immunized with[b]:					
	KOS	186	MP	RS1G25	R50BG10	RH1G44
HSV-1 KOS	52.0	47.1	60.7	40.4	51.5	54.2
HSV-2 186	9.9	26.2	14.4	7.9	13.4	11.2
HSV-1 MP	70.6	68.6	74.8	68.7	69.3	71.2
RH1G44	47.2	44.5	57.6	39.4	46.4	51.5
RS1G25	50.7	52.6	52.9	47.2	52.9	50.3
R50BG10	20.5	25.8	32.3	17.6	23.8	25.2
None	4.6	9.6	6.2	1.5	6.4	3.1

[a] Target cells were infected with the designated virus strain at a MOI of 2.5 or mock infected, labeled with 200 μCi of ^{51}Cr, and harvested 14 h postinfection. Lymphocyte cytotoxicity was determined in a 5-h ^{51}Cr release assay at 37°C, at an effector-to-target cell ratio of 40:1.

[b] Values represent the percent specific ^{51}Cr release from target cells.

TABLE 4. Effect of coinfection of B6/WT-3 target cells with HSV-1 KOS and either HSV-2 186 or R50BG10 on their susceptibility to lysis by anti-HSV CTLs

Target cells (B6/WT-3) infected by[a]:	MOI	% Specific ^{51}Cr release[b]			
		Expt 1	Expt 2	Expt 1	Expt 2
HSV-1 KOS	2.5	22.9	49.5	14.5	41.9
HSV-2 186	2.5	3.7	10.9	4.1	13.0
R50BG10	2.5	10.6	22.7	8.1	16.0
HSV-1 KOS + HSV-2 186	2.5 2.5	6.0	10.5	5.3	10.5
HSV-1 KOS + R50BG10	2.5 2.5	8.4	19.1	6.6	14.5
None		1.6	5.2	1.0	6.0

[a] Target cells were infected with the designated virus strain or mock infected, labeled with 200 μCi of ^{51}Cr, and harvested 14 h postinfection.

[b] Lymphocyte cytotoxicity was determined in a 5-h ^{51}Cr release assay at 37°C, at an effector-to-target cell ratio of 40:1.

HSV-1-infected cells upon superinfection with HSV-2 is not due to a lack of synthesis of HSV-1 glycoproteins.

DISCUSSION

The results presented above have focused on the lower susceptibility of HSV-2-infected cells to lysis by anti-HSV CTLs. In our system, the levels of lysis by anti-HSV CTLs are lower for HSV-2 186-infected cells than for HSV-1 KOS-infected cells, even though similar levels of viral glycoproteins were expressed on the surfaces of HSV-2-infected B6/WT-3 cells and HSV-1-infected cells (5). We therefore used HSV-1 × HSV-2 recombinants with defined regions of HSV-2 genome (Table 1) to determine which segment of HSV-2 DNA may code for the function(s) involved in the reduced susceptibility of HSV-2-infected cells to lysis.

The B6/WT-3 target cells infected with the HSV-1 × HSV-2 recombinants RH1G44 and RS1G25 were as susceptible to lysis as HSV-1 KOS-infected cells. However, B6/WT-3 target cells infected with the HSV-1 × HSV-2 recombinants R50BG10, A7D, and C4D exhibited low levels of lysis by anti-HSV CTLs, as was observed with HSV-2 186-infected cells. These results indicate that the HSV-2 function(s) that affects cell susceptibility to lysis by anti-HSV CTLs maps within the HSV-2 DNA sequences of R50BG10, A7D, and C4D. The recombinant R50BG10 was isolated from cells transfected with HSV-1 *cs*50B DNA and HSV-2 G DNA digested with the restriction enzyme *Hpa*I (33) and contains HSV-2 sequences to the right of 0.82 map unit on the HSV genome. The recombinant A7D was generated by rescuing a *ts* mutant of HSV-1 17 strain resistant to phosphonoacetic acid (PAA) with PAA-sensitive HSV-2 (GP6) virions, whereas the recombinant C4D was generated by rescuing a *ts* mutant of HSV-1 (HFEM)PAA^r with HSV-2 (186)PAA^s virions (17). The recombinant C4D was shown to contain HSV-2 DNA sequences from 0.0 to 0.28 and from 0.72 to 1.0 map unit, whereas the recombinant A7D contained HSV-2 DNA sequences from 0.78 to 1.0 map unit. It is not known whether A7D and C4D contain additional HSV-2 DNA not detectable by the restriction enzyme analysis of the intertypic recombinant DNA. Two HSV-specific glycoproteins have been identified which map in the S component of HSV-1 genome: gD and gE (19, 30). Glycoproteins gD and gE of HSV-2, as well as an additional 92K HSV-2 glycoprotein, also map in the S component of HSV-2 genome (15, 16). This glycoprotein of HSV-2 has been designated gG (B. Roizman, personal communication). However, the specific involvement of gD, gE, or the 92K to 120K glycoprotein in the lowered susceptibility of HSV-2-infected cells to lysis by anti-HSV CTLs could not be determined from these studies. We also cannot exclude the possibility that other functions affecting the target cell susceptibility are encoded in this region of DNA.

The B6/WT-3 target cells infected with HSV-1 MP were lysed as efficiently by anti-HSV CTLs as were HSV-1 KOS-infected cells. The HSV-1 MP strain causes polykaryocyte formation of infected cells, and this fusion capability of HSV-1 MP may be involved in the substantially higher values of percent specific ^{51}Cr release obtained with HSV-1 MP-infected cells seen in some experiments (Table 3). The results presented here demonstrate that gC is not essential for the induction of highly reactive CTLs. The CTLs induced by HSV-1 MP were highly efficient at lysing target cells infected with HSV-1, HSV-2, or HSV-1 × HSV-2 recombinants. Since HSV-1 MP does not specify gC, the CTL population induced by the virus must be directed against cross-reactive determinants on gA/B, gD, or gE. As reported previously (5), this system for the induction of CTLs appears to induce predominantly cross-reactive populations of CTLs. A secondary splenic CTL system has been described in which the secondary stimulation of lymphocytes by either homologous or heterologous viruses delineates both type-specific and cross-reactive CTL populations (11); the type-specific CTL population recognizes type-specific determinants on gC, whereas the cross-reactive CTL population recognizes determinants on other glycoprotein species. The primary system used in this study does not detect significant levels of type-specific CTLs, presumably reflecting the relative unimportance of gC in this system. This apparent dichotomy between the two systems may be due to differences in the local response to HSV in the spleen and the popliteal lymph node. The HSV-1 × HSV-2 recombinants were also efficient at inducing CTLs, and these CTL populations contained predominantly cross-reactive CTLs. Recent studies (34), however, have shown that HSV-1-

TABLE 5. Effect of superinfection with HSV-2 of HSV-1-infected cells on the susceptibility to lysis by anti-HSV CTLs

Expt no.	Duration of infection[a] (h) by:		% Specific ^{51}Cr release[b]
	HSV-1 (KOS)	HSV-2 (186)	
1	14	–	40.9
	–	14	10.9
	14	14	12.9
	14	2	38.9
2	14	–	41.1
	–	14	11.8
	14	14	19.1
	14	4	41.6
3	14	–	40.4
	–	14	10.8
	14	14	7.5
	14	2	37.8
	14	8	14.0

[a] Target cells (B6/WT-3) were infected with HSV-1, HSV-2, or both for various time periods, labeled with 200 μCi of ^{51}Cr, and reacted with CTLs generated to HSV-1.

[b] Lymphocyte cytotoxicity was determined in a 5-h ^{51}Cr release assay at 37°C, at an effector-to-target cell ratio of 50:1.

coded gC possess antigenic determinants cross-reactive with gC of HSV-2.

In an attempt to determine the nature of the function(s) affecting the susceptibility of HSV-2-infected cells to lysis by CTLs, B6/WT-3 target cells were coinfected with HSV-1 KOS and either HSV-2 186 or R50BG10. The coinfected B6/WT-3 cells exhibited the HSV-2 level of susceptibility to lysis by anti-HSV CTLs. Varying the MOI of HSV-1 KOS and HSV-2 186 did not alter the susceptibility of the coinfected cells to lysis by HSV CTLs. In addition, increasing the MOI of HSV-1 KOS to a level 10-fold higher than that of HSV-2 186 did not significantly increase the susceptibility of the coinfected cells to lysis by anti-HSV CTLs. These results indicate that the HSV-2 function(s) is dominant in cells coinfected with HSV-1 KOS and HSV-2 186. It is possible that some differential alteration of membrane permeability or an altered configuration of the HSV-2 glycoproteins and cellular *H-2* antigens could result in a reduced susceptibility to lysis by CTLs. Since the HSV-2-infected cells are as susceptible to lysis by antibody and complement as are the HSV-1-infected cells (5), and since glycoproteins of both types are synthesized during coinfection, the data provide a distinction between T cell-mediated cytolysis and antibody-dependent complement-mediated lysis of HSV-infected cells.

The results reported here indicate that the reduced susceptibility of HSV-2-infected cells to lysis by anti-HSV CTLs maps to a function contained within the S component of the HSV-2 genome. Whether this reduction in susceptibility is associated with a particular glycoprotein species mapping within this region, or with some other HSV-2-specific function, has not been determined. Studies are in progress to examine both the specific involvement of HSV glycoproteins on the susceptibility to lysis by anti-HSV CTLs and the relative effects of infection with HSV-1 and HSV-2 strains and recombinant viruses on the qualitative and quantitative expression of *H-2* antigens at the cell surface.

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