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Cell-Mediated Immunity Against Herpes Simplex Induction of Cytotoxic T Lymphocytes

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The conditions required for the induction of both primary cytotoxic T lymphocytes (CTL) in vivo and secondary CTL in vitro against herpes simplex virus type 1 (HSV-1)-infected cells were defined. Primary CTL responses occurred only in mice exposed to infectious HSV-1. These responses, which were shown to be mediated by T lymphocytes, peaked at 1 week and had disappeared by 2 weeks after infection. The level of primary cytotoxicity was enhanced by treatment of mice with cyclophosphamide before infection. Secondary in vitro CTL responses were more pronounced and were induced by some forms of inactivated virus as well as by infectious HSV-1. Thus, both ultraviolet light- and glutaraldehyde-inactivated preparations of HSV-1 induced CTL, but heat-inactivated and detergent-extracted antigens failed to do so. The reasons for the differing efficiency of infectious and noninfectious HSV-1 for induction of CTL are discussed.

Several persuasive communications have established the concept that recovery from many viral infections is essentially a function of cell-mediated immunity (CMI) carried out by T cells (1, 3, 25, 30). Numerous functional subsets of T cells are now recognized which in mice, at least, can also be identified by surface antigenic differences (6, 18). Of the functional subsets, much recent attention has been focused on cytotoxic T lymphocytes (CTL), since it appears that such cells may act in immunity by destroying virus-infected cells early after infection, thereby curtailing viral spread (37, 38). In mediating their cytotoxicity, at least in the mouse system, CTL need to recognize not only virus but also some product of the major histocompatibility complex (MHC), the phenomenon referred to as H-2 restriction (7). Since CTL are important in immunity, it is essential to understand how best to induce such cells. Thus, any immunization protocol should induce or prime for the establishment of CTL so that upon infection, recovery can occur rapidly and preferably subclinically. The induction as well as the effector activity of CTL against certain viral antigens also requires the presentation of virus in association with an MHC product (7, 12, 14). However, it is not yet clear whether induction results from the direct presentation of viral antigen and MHC product to the CTL precursor or whether induction occurs indirectly, and perhaps nonspecifically, by triggering the release of mediators from helper T cells (26, 34).

Although it has been reported that mice, upon infection by herpes simplex virus (HSV), may produce CTL (28, 32), such responses are weak and have not always been observed (29). It is especially important to define how best to induce cell-mediated responses to herpesvirus antigens, since this group of viruses is considered a good example of viruses in which protective immune mechanisms are largely antibody independent (3, 30). Furthermore, since it has been demonstrated that inactivated herpesvirus vaccines may be oncogenic (8, 9), vaccines against herpesvirus for future use in humans must be of the subunit type. Whether or not such preparations can induce CMI needs to be defined. In the present communication, we describe conditions required for the induction of CTL responses against HSV type 1 (HSV-1) in mice. Later communications will deal with CTL induction by viral subunit antigens.

MATERIALS AND METHODS

Cells and viruses used. Strain L929 cells (H-2^k) were obtained from Flow Laboratories and were grown in McCoy 5A medium supplemented with 5% calf serum. Strain BALB/c 3T3 clone A31 (H-2^d) cells (abbreviated A31) were obtained from Ray Tennant, Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tenn., and were grown in McCoy 5A medium supplemented with 10% fetal calf serum (FCS). Cell strain HEp-2, obtained from Flow Laboratories, was cultured in McCoy 5A plus 10% donor calf serum. HSV-1 strain KOS was grown in HEp-2 cells by infecting cells at low multiplicity and harvesting cell-associated virus as described previously (4). Viral stocks used had an infectivity titer of $10^{8.5}$ plaque-

forming units (PFU) per ml. Vaccinia virus was obtained from P. C. Doherty, Wistar Institute, and grown in L cells; this virus had an infectivity titer of 10^7 PFU/ml.

Mouse immunization and preparation of spleen cells. Young female C3H mice (H-2^k) and BALB/c mice (H-2^d) were obtained from Cumberland View Farms, Clinton, Tenn. Animals were infected intraperitoneally with 0.2-ml inocula containing various amounts of HSV-1 KOS or inactivated material. One group of mice received ultraviolet light (UV)-inactivated virus emulsified in Freund complete adjuvant (Difco Laboratories, Detroit, Mich.). At various times after infection or immunization, mice were killed by cervical dislocation and their spleens were removed aseptically. Single-cell suspensions were prepared by teasing cells through 80-mesh sieves into Hanks balanced salt solution containing 5% FCS (HBSS). After one wash, erythrocytes were lysed by brief exposure at 37°C to 0.83% NH_4Cl. Cells were then washed in HBSS twice more, the cell aggregates were removed, and the viable cells were enumerated. To measure cytotoxicity, spleen cells were adjusted to various concentrations in RPMI 1640 containing 7% FCS, penicillin (100 U/ml), and streptomycin (100 μg/ml) and buffered to pH 7.2 with *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (RPMI-HEPES).

For in vitro culture, spleen cells were adjusted to 2×10^6/ml in RPMI 1640 containing 10% FCS, 2 mM glutamine, penicillin (100 U/ml), streptomycin (100 μg/ml), and 5×10^{-5} M 2-mercaptoethanol and added to plastic tissue culture plates at a cell density of 10^6 per cm^2 surface area. The spleen cell cultures were incubated in a humidified CO_2 (5%) incubator usually for 5 days, after which time cells were harvested, washed twice in medium, and adjusted to the desired concentration for cytotoxicity in RPMI-HEPES. For in vitro stimulation, either viable HSV-1 or an inactivated preparation was added directly to culture dishes containing spleen cells.

Cell fractionation. Glass wool columns were prepared by loosely packing wool (Pyrex, Corning, N.Y.) in 10-ml syringes. Columns were equilibrated with RPMI-HEPES at 37°C, and 50×10^6 spleen cells were slowly filtered through. Nonadherent cells were collected. Separation of spleen cells on nylon wool was performed as described by Julius et al. (17). Monoclonal antithymocyte 1.2 serum was kindly donated by Jon Sprent, Wistar Institute. Spleen cells (10^7/ml) in RPMI-HEPES were reacted with a 40 μl of a 1/10 dilution of antiserum for 30 min at 4°C and then washed and reacted with a 1/6 dilution of guinea pig serum, previously adsorbed with normal mouse spleen cells. After treatment with complement for 30 min at 37°C, the surviving cells were enumerated and used as an effector population. The method of treatment with anti-immunoglobulin serum plus complement to remove B cells was identical to that described for antithymocyte 1.2 serum. Thus, 10^7 spleen cells were reacted for 30 min with a 1/5 dilution of heat-inactivated rabbit anti-immunoglobulin serum (Cappel Laboratories, Evanston, Pa.) and washed; this procedure was followed by complement treatment as described above.

Virus inactivation. For UV treatment, virus ($10^{8.5}$ PFU/ml) suspended in phosphate-buffered saline was exposed at a distance of 9 cm from two G15T8 germicidal bulbs (General Electric Co., Waterford, N.Y.) for varying times. One sample was taken for measurement of infectivity as described elsewhere (4). Heat-inactivated virus was prepared by holding virus in McCoy medium at 56°C for 60 min. Glutaraldehyde-inactivated stimulator cells were prepared by taking L cells (5×10^6) infected at a multiplicity of infection of 1 15 h previously with HSV-1 and exposing them for 15 s to 0.1 M glutaraldehyde. After this time the fluids were immediately diluted with 50 volumes of culture medium, washed twice more, resuspended to 200 μl, and used to stimulate 25×10^6 primed spleen responder cells.

Detergent extraction of virus antigens was accomplished by resuspending HSV-1-infected HEp-2 cells (infected at a multiplicity of infection of 10 PFU/cell and harvested at 24 h postinfection) to a concentration of 10^7 cells/ml in water. The cell suspension was sonicated for 2 min (Heat Systems, Ultrasonics, Inc., Plainview, N.Y.) at maximal power followed by the addition of sodium deoxycholate and Tween 40 to a final concentration of 1%. The extract was incubated at 37°C for 1 h and then centrifuged at $100,000 \times g$ for 1 h. The supernatant represented the solubilized HSV-1 antigen extract used in these studies.

Cyclophosphamide treatment. Groups of C3H and BALB/c mice were injected intraperitoneally with 100 mg of cyclophosphamide per kg 48 h before infection with 10^8 PFU of HSV-1 KOS. Cyclophosphamide-treated and control (infected but untreated) mice were killed at 6 days after infection for measurement of primary cytotoxicity.

Cytotoxicity assays. L or A31 cells (5×10^6 to 10×10^6) were suspended in McCoy 5A medium and infected with HSV-1 at a multiplicity of infection of 1 for 60 min at 37°C with occasional agitation in an atmosphere of 5% CO_2 in air. After infection, cells were washed once with RPMI-HEPES and suspended in 1 ml of RPMI-HEPES containing 100 μCi of $Na_2{}^{51}CrO_4$ (New England Nuclear Corp., Boston, Mass.). After incubation for 1 h at 37°C in an atmosphere of 5% CO_2, cells were washed three times with medium and suspended at 10^5/ml in RPMI-HEPES. Uninfected L cells were labeled simultaneously. Vaccinia-infected cell targets were prepared by infecting L cells at a multiplicity of infection of 0.1 14 h before labeling with ^{51}Cr. All target cells were added as 100-μl volumes (10^4 cells) to U-shaped microtiter plates.

The effector cells were enumerated for viable cells, suspended in RPMI-HEPES, and added to microtiter plates in 100-μl volumes. Microtiter plates were centrifuged at $200 \times g$ for 1 min before incubation. Different effector-to-target ratios were carried out, and all assays were performed in quadruplicate.

Cytotoxicity assays were begun 3 h after the initiation of infection with HSV-1 and were carried out for 7 h in a 5% CO_2 humidified incubator. The antivaccinia assays were performed for 16 h of incubation. At the end of incubation, 50% of the well contents was harvested for measurement of radioactivity. Standard errors were low (<4%), and results are reported as mean percent specific release values computed according to the following formula: percent specific ^{51}Cr

release = [(effector cell release − media control release)/(total releasible chromium − media control release)] × 100. The total releasible ^{51}Cr was obtained by exposing target cells to 1% Triton X-100.

RESULTS

Primary induction of CTL. C3H mice (H-2^k), upon infection with HSV-1, generated CTL (Fig. 1). This response peaked at 6 to 8 days postinfection and was almost undetectable by 12 days postinfection. Two levels of specificity characteristic of all murine T-cell-mediated antiviral cytotoxicities (7) were observed, with the CTL being virus specific and only detectable with homologous histocompatible targets. Thus, cytotoxicity was undetectable against either HSV-1-infected A31 (H-2^d) targets or syngeneic L-cell (H-2^k) targets infected with vaccinia virus (Fig. 1). Further evidence that the cytotoxicity observed was T-cell mediated came from experiments showing that the cells were nonadherent to nylon and glass wool and were destroyed by treatment with antithymocyte 1.2 serum plus complement (Table 1). Treatment with anti-immunoglobulin serum plus complement failed to reduce the level of cytotoxicity (Table 1).

The level of primary cytotoxicity resulting from HSV-1 infection can be considered weak compared with that observed with several other viruses (7). We have observed similar low levels of cytotoxicity against HSV-1 in other strains of mice [BALB/c and (BALB/c × C_3H)F_1] and in mice infected with more virulent strains of HSV-1 (data not shown). Furthermore, in C3H mice repeatedly exposed (two to six times) to HSV-1, we have not observed specific cytotoxicities above 25% at a 100:1 effector-to-target cell ratio with spleen cells taken 8 days after viral exposure. However, as observed with some other T-cell responses (13, 21), pretreatment of mice with cyclophosphamide (100 mg/kg) 2 days before infection raised the level of cytotoxicity more than twofold (Table 1).

To induce primary cytotoxicity against HSV-1, sufficient infectious virus needed to be administered (Table 2). Thus, inoculation of less than 10^6 PFU of infectious virus failed either to stimulate a primary response or to sensitize animals for a secondary response in vitro. In addition, virus inactivated by heat, UV, or detergent-extracted material all failed to elicit a detectable primary T-cell cytotoxic response (Table 2).

Induction of secondary CTL responses in vitro. In contrast to the low level of cytotoxicity observed in mice after primary and secondary in vivo infection with HSV-1, spleen cells from mice primed in vivo with HSV-1 developed high levels of cytotoxicity upon secondary restimulation in vitro with HSV-1 (Fig. 2). Percent specific

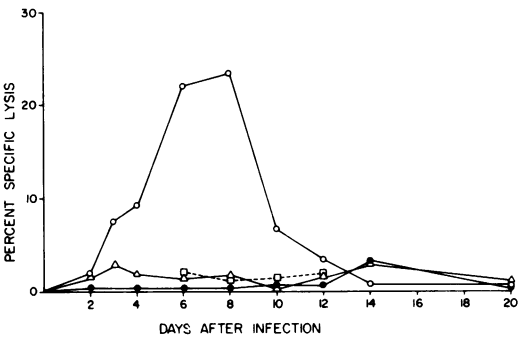

FIG. 1. *Development of CTL against HSV-infected L cells (○), virus-A31 cells (△), uninfected L cells (●), and vaccinia virus-infected L cells (□) in C3H mice infected intraperitoneally with 10^8 PFU of HSV-1.*

TABLE 1. *Primary induction of antiherpes CTL in C3H mice and nature of the effector cells*

Effector cell population	% Specific lysis[a]			
	L cells + HSV	L cells	A31 cells + HSV	L cells + vaccinia
Nonimmune	0	0.8	0.6	0
Immune[b]	25	5.1	13.0	1.4
Glass wool nonadherent	25.1	1.0	6.2	ND[c]
Nylon wool nonadherent	49.6	4.2	7.9	ND
Antithymocyte 1.2 serum + complement	2.7	5.4	0	ND
Complement	27	2.9	6.3	ND
Anti-immunoglobulin + complement	29.2	ND	ND	ND
Cyclophosphamide pretreated[d]	54	4.4	13	4.3

[a] Means of quadruplicate assays at effector-to-target cell ratio of 100:1. Assay time, 7 h. Standard errors were less than 4%.

[b] Pooled spleen cells from two C3H mice infected 6 days previously with 10^8 PFU of HSV-1.

[c] Not done.

[d] Pooled spleen cells from three mice treated 2 days before infection with 10^8 PFU of HSV-1 plus 100 mg of cyclophosphamide per kg. Animals were killed at 6 days postinfection.

TABLE 2. *Primary induction of CTL and in vitro secondary response in mice immunized with active and inactivated HSV-1*

Immunization protocol[a]	% Specific lysis against L-HSV targets	
	Day 7 CTL, 100:1	Memory CTL,[b] 25:1
10^8 PFU of HSV	24.8	72.8
10^6 PFU of HSV	22.8	77.0
10^4 PFU of HSV	3.2	5.0
10 min of UV, HSV	2.1	10.5
60 min of heat, 56°C (10^2 PFU)	1.8	3.4
10 min of UV, HSV + FCA[c]	0	1.9
Detergent-extracted HSV-infected cells	0.9	5.3
Nonimmunized	1.6	3.0

[a] Groups of four C3H mice were infected intraperitoneally with virus or inactivated preparations.

[b] Determined by culturing 25 × 10^6 spleen cells with 10^8 PFU of HSV for 5 days and measuring CTL.

[c] Animal killed at day 20 for measurement of primary CTL and memory response. FCA, Freund complete adjuvant.

cytotoxicities were always greater than 60% at an effector-to-target cell ratio of 25:1, and in some experiments were 95%. Priming for secondary cytotoxicity was present by day 4, as evidenced by specific cytotoxicity against syngeneic HSV-1-infected targets of 31% as compared with 9.8% by secondary restimulated and unstimulated immune spleen cells, respectively. Peak levels of cytotoxicity were observed in spleen cells from mice exposed for 6 days or more to HSV-1 before secondary restimulation (Fig. 2). These peak levels of cytotoxicity persisted for at least 6 months (longest time tested). HSV-1-stimulated primed spleen cells showed some cytotoxicity against uninfected syngeneic L-cell targets (Fig, 1, Table 3). This level of cytotoxicity was variable (8 to 30%) and was most marked with HSV-1-primed cells. In addition, the level of cytotoxicity against uninfected cells was greater with antigen-stimulated spleen cells than those cultured in the absence of antigen stimulation. Nonspecific killing of uninfected cells by antigen-stimulated primed cells has been noted in other viral systems (2, 24). An adequate explanation for the phenomenon has not been forthcoming.

Finally, the secondary cytotoxic activity showed that H-2 restriction and treatment with antithymocyte 1.2 serum plus complement destroyed the cytotoxicity (Table 3), indicating that the response was T-cell mediated. Some cytoxicity was always apparent against allogeneic HSV-infected targets, but this level of cytotoxicity was similar to that observed against uninfected L cells.

As an initial approach to further investigate

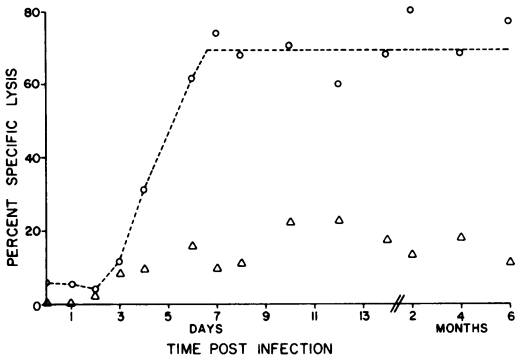

FIG. 2. *Time course of generation of CTL precursors in C3H mice infected with HSV strain KOS. The response of spleen cells stimulated in vitro against HSV-infected L cells* (○) *and uninfected L cells* (△) *at a 25:1 effector-to-target cell ratio.*

the antigens and conditions required for CTL induction against herpesviruses, the effect of viral dose and form of inactivation were investigated. The induction of CTL activity in 25 × 10^6 spleen cells required more than 10^4 PFU of infectious virus (Table 3). Furthermore, induction could be achieved with UV-inactivated virus, although with prolonged UV treatment the preparations became less immunogenic. In addition, induction occurred with glutaraldehyde-inactivated virus but not with heat-inactivated material. Finally, it has not yet proven possible to induce CTL with soluble herpesvirus antigens extracted with detergents, although preliminary experiments have shown induction of CTL with soluble antigens incorporated into vesicles (M. J. B. Lawman, B. T. Rouse, and R. J. Courtney, unpublished data).

DISCUSSION

It is commonly considered that immunity, especially recovery, from herpesvirus infections is primarily a function of CMI (for reviews, see references 1, 2, 25, 30). A corollary to this notion is that any antiherpesvirus vaccination protocol must stimulate adequate T-cell function. Since doubts exist regarding the use of live or inactivated whole herpesvirus vaccines in humans, future vaccines must be of the subunit type and should contain antigens that induce protection. The primary aim of our present study was to define the conditions under which mice generate T-cell cytotoxic responses to HSV-1, with such studies forming a prelude to the future evaluation of isolated herpesvirus proteins for their ability to stimulate CMI. Our results show that upon infection with a laboratory strain of HSV-1, a primary CTL response occurred, but this response was weak and short-lived. Its demon-

TABLE 3. *Induction of cytotoxicity in primed spleen cells by active and inactivated HSV-1*

Spleen cell population[a]	Stimulant	% Specific lysis[b]			
		L cells + HSV-1	L cells	A31 + HSV-1	L cells + vaccinia
Normal	None	0	1.2	1.5	0.8
Normal	10^8 PFU of HSV	1.7	0	0.9	0
Immune	None	7.8	5.0	6.2	6.0
Immune	10^8 PFU of HSV	70	13.9	10.9	14.7
Immune	10^6 PFU of HSV	28.6	3.9	ND[c]	ND
Immune	10^4 PFU of HSV	7.5	7.2	ND	ND
Immune	2 min of UV, HSV ($10^{3.8}$ PFU)[d]	74	16.0	12.0	ND
Immune	5 min of UV, HSV ($10^{2.0}$ PFU)	76	13.0	12.5	ND
Immune	10 min of UV, HSV (<10 PFU)	66	12.2	11.5	ND
Immune	20 min of UV, HSV (<10 PFU)	18.6	7.2	3.1	ND
Immune	60 min of heat 56°C ($10^{2.0}$ PFU)[e]	8.1	7.5	2.1	ND
Immune	Glut inactivated (<10 PFU)[f]	61.3	18.7	16.0	ND
Immune	DOC extracted (<10 PFU)[g]	4.0	0	ND	ND
Immune	10^8 PFU of HSV, antithy 1.2[h]	6.5	ND	ND	ND

[a] Either 25×10^6 pooled spleen cells from nonimmunized C3H mice or from mice 4 weeks after infection with 10^8 PFU of HSV-1 (immune) were cultured in vitro for 5 days with different stimulants.

[b] Mean of quadruplicate assays at effector-to-target cell ratios of 25:1 for 7 h. The standard errors were less than 4%.

[c] ND, Not done.

[d] 10^8 PFU of HSV virus inactivated by UV for 2 min before addition to spleen cultures. The figures in parentheses refers to the infectivity remaining.

[e] 10^8 PFU of virus inactivated by heat at 56°C for 60 min.

[f] Glutaraldehyde-inactivated HSV-infected L-cell stimulant.

[g] HSV-infected cells extracted with detergent as described in the text.

[h] Immune HSV-stimulated spleen cells treated with antithymocyte 1.2 serum plus complement to destroy T cells.

stration required high effector-to-target cell ratios (100:1), and even with such ratios specific cytotoxicity did not exceed 25% at its peak. Compare this with viruses such as vaccinia, where similar levels of cytotoxicity are observed at effector-to-target cell ratios of 5:1 (14; Lawman and Rouse, unpublished data). Previous investigators working with murine cytotoxic responses to HSV have either failed to observe the phenomenon (29) or have needed to expose animals to virus repeatedly (32) or culture cells in vitro for a period of 3 days in order to demonstrate cytotoxicity (28). Why HSV-1 infection gives rise to a poor CTL response was not resolved, but it seems not to be associated with viral virulence, since we have observed similar poor responses after infection with more virulent strains (Lawman et al., unpublished data). The most likely explanation may be that some suppressor mechanism, antibody, T-cell, or macrophage mediated, was regulating the extent of CTL activity. Indeed, the observation of Pfizenmaier et al. (28) of CTL responses only after in vitro culture could be explained by the disappearance of a suppressor cell population, as could our own observation that the primary CTL response was considerably enhanced after pretreatment with cyclophosphamide. Others have interpreted the ability of cyclophosphamide to enhance T-cell activity to be by its selective effects either on suppressor cells (13) or on antibody production (21, 33), the latter serving to block T-cell activity as observed in several tumor systems (review in reference 15). That HSV-1 may stimulate suppressor macrophages has been suspected by others (20), but our failure to observe enhancement of lytic activity after the removal of macrophages on glass wool columns fails to support a suppressive role of macrophages at least in the effector cell population. Clearly, more studies are needed to unravel the reasons for a sluggish CTL response to HSV infection. A solution to the question of whether some suppressor mechanism is involved may come from studies with alloantisera of defined specificity to selectively deplete certain cell populations (18).

The kinetics of the cytotoxicity response, the fact that it was virus specific and H-2 restricted, and the fact that the effector cells were nonadherent to nylon wool and expressed the thymocyte 1.2 alloantigen were all evidence that the nature of the response was T-cell mediated. We were only able to obtain such responses in mice exposed to infectious HSV-1. Thus, neither UV-inactivated heat-inactivated, nor detergent-ex-

tracted viral antigens could, under the conditions investigated, elicit a primary response. Furthermore, such inactivated preparations failed to prime mice for secondary in vitro cytotoxicity, a more sensitive test for antiherpes CTL in mice (discussed below). The failure of inactivated viral preparations to stimulate CMI responses detectable by blastogenesis as well as cytotoxicity has been noted with some other viruses (5, 11, 19), although many viruses do elicit potent CTL responses even when inactivated (12, 14, 27, 31, 35). Thus, inactivated vaccinia (14) and Sendai (27) viruses are equal to the active virus at eliciting CTL responses, and inactivated rabies virus also induces CMI (35). Since these viruses are enveloped, it was suggested that this efficacy at stimulating CTL when inactivated may be because they are able to fuse into the membrane of stimulator cells and make the interaction with H-2 antigens essential for the induction of CTL responses (14). This hypothesis, however, has not been substantiated and was made unlikely by the observation of Mescher et al. (22) with a Sendai virus system in which stimulating primed F_1 cells with virus associated with stimulator membranes of one haplotype only induced effector cells capable of lysing viral cell targets of the same haplotype. If fusion had occurred with the responder F_1 cells, effectors capable of lysing both haplotype targets would have been anticipated. Failure to cause fusion, however, was also considered to be the explanation for the failure of inactivated influenza to elicit CTL (5), although some workers have demonstrated primary CTL responses with inactivated influenza antigens (10). Although it is known that active HSV may fuse with the cell membrane upon infection (16, 23), whether or not inactivated preparations may do so has not been reported and is under investigation in our laboratory. It may be that induction of CTL in vivo with inactivated viral antigens will require either a suitable adjuvant or a preparation such as a liposome that may permit suitable presentation of antigen to responder cells.

Whereas primary CTL responses to HSV-1 were ephemeral and only obtained after infection with live virus, potent CTL responses were achieved upon secondary stimulation of primed spleen cells in vitro. Moreover, some but not all forms of inactivated virus could also elicit secondary responses in vitro. Such responses were induced with UVand glutaraldehyde-inactivated preparations, but not with heat-inactivated or detergent-extracted antigens. Furthermore, prolonged inactivation with UV also markedly reduced its ability to induce CTL in vitro. Why some forms of inactivation render herpesvirus antigens unable to induce CTL in vitro was not determined, but some form of protein denaturation was the explanation considered most likely. This denaturation was not complete because heat-inactivated herpesvirus preparations were still antigenic in that they stimulated blastogenesis of T cells, induced antibody production, and reacted with specific antiherpesvirus antibody in vitro (36; unpublished observations). We have presumed that the nonstimulatory herpesvirus preparations were altered in their tertiary structure such that they were unable to present these determinants in optimum form to CTL precursors or accessory cells. Except in the case of influenza virus (39), presentation of viral proteins in conjunction with H-2 antigens is known to be required to induce CTL in vitro; for such induction to occur, viral antigen and H-2 must be present in the same membrane (11). Similarly, induction of CTL against Sendai virus antigens can only be demonstrated when viral antigens and H-2 antigens are combined in the same artificial membrane, and even in these circumstances the efficiency of CTL induction is less than that by virus-infected viable cells (12). It may be that certain forms of viral inactivation yield products unable to stimulate because these fail to combine optimally with some H-2 product. Whether they fail to induce CTL because the virus–H-2 combination then fails to trigger precursor CTL directly or because the combination fails to trigger the release of a factor from a vital accessory cell is a topic in need of elucidation.

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