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Herpes Simplex Virus-Infected Human Fibroblasts Are Resistant to and Inhibit Cytotoxic T-Lymphocyte Activity

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We examined the ability of human anti-herpes simplex virus (HSV) cytotoxic T lymphocytes (CTL) to lyse autologous human fibroblasts infected with HSV. In contrast to HSV-infected human Epstein-Barr virus-transformed B cells (LCL), which were lysed by HLA-restricted anti-HSV CTL, autologous fibroblasts infected with HSV were resistant to lysis. This resistance was not due to a lack of infectivity or production of HSV proteins since greater than 90% of the cells were infected and expressed abundant levels of viral proteins. HSV-infected human fibroblasts were also tested for susceptibility to lysis by alloantigen-specific CTL. Although allogeneic LCL and uninfected allogeneic fibroblasts were killed, human fibroblasts infected with HSV demonstrated a time-dependent resistance to lysis by alloantigen-specific CTL. HSV-infected human fibroblasts were not resistant to all forms of cell-mediated cytotoxicity since they were sensitive to antibody-dependent cellular cytotoxicity. Although one may suspect that the resistance of HSV-infected human fibroblasts to anti-HSV CTL and alloantigen-specific CTL-mediated lysis was due to a lack of major histocompatibility complex expression, Confer et al. (Proc. Natl. Acad. Sci. USA 87:3609–3613, 1990) previously demonstrated that incubation of human natural killer and lymphokine-activated killer cells with monolayers of human fibroblasts infected with HSV "disarmed" the killers in that they were unable to lyse sensitive target cells. We extend their results and show that incubation of anti-HSV CTL or alloantigen-specific CTL with uninfected fibroblasts did not affect their lytic activity, whereas CTL incubated with HSV-infected fibroblasts for 2 to 6 h rendered the CTL incapable of lysing their normally sensitive target cells. Indeed, human fibroblasts infected for merely 2 h with HSV were able to profoundly inhibit the cytotoxic activity of alloantigen-specific CTL. Thus, HSV-infected human fibroblasts are not inherently resistant to lysis by anti-HSV CTL or alloantigen-specific CTL, but rather contact of CTL with HSV-infected fibroblasts resulted in inactivation of the CTL. The inactivation of CTL appears to be HSV specific since incubation of alloantigen-specific CTL in sandwich assays with fibroblasts infected with HSV type 1 (HSV-1) or HSV-2 resulted in inactivation, whereas incubation of CTL with fibroblasts infected with adenovirus or vaccinia virus had no effect. Further, although incubation of alloantigen-specific CTL in sandwich assays with HSV-infected fibroblasts resulted in inhibition of CTL activity, exposure of CTL in Transwell cultures to cell-free supernatant from HSV-infected fibroblasts did not mediate this inhibitory effect. Thus, the inhibition of CTL by HSV-infected fibroblasts is not mediated by a soluble factor but rather appears to require cell-to-cell contact. Gaining an understanding of the mechanism by which HSV-infected human fibroblasts inhibit CTL activity may be relevant to our understanding of virus-induced immunosuppression and how viruses escape from immune surveillance and provide insight into inactivation and anergy of mature cytotoxic effector cells.

A number of viruses are known to establish persistent infections in immunocompetent individuals. To achieve this, a virus must persist within cells in the host, and these virus-infected cells must evade immune surveillance and destruction, especially from antiviral cytotoxic T lymphocytes (CTL). To avoid detection and destruction by antiviral CTL, viruses have evolved numerous mechanisms. One example of this is the downregulation of major histocompatibility complex (MHC) expression that occurs in cells infected with certain adenovirus subtypes (29). More recently, it was shown that viruses may also escape immune surveillance by mutating crucial T-cell epitopes recognized by CTL. This was demonstrated for lymphocytic choriomeningitis virus both in vivo (17) and in vitro (1) and for human immunodeficiency virus (15). Alternatively, viruses may be immunosuppressive by directly or indirectly interfering with cells of the immune system (12). Human immunodeficiency virus, for example, infects $CD4^+$ T cells, which results in their depletion and subsequent immunosuppression in infected individuals.

Cell-mediated immunity is known to play an important role in controlling the spread and severity of herpes simplex virus (HSV) infection in humans. Indeed, the severity of infection with HSV is inversely correlated with the competency of the host cellular immune response (6). The role of T cells in controlling HSV infections is further implicated by the serious, sometimes life-threatening, primary or recurrent infections that can develop in immunocompromised individuals, particularly transplant recipients treated with immunosuppressive drugs, such as cyclosporin A (14, 16, 21), and individuals with AIDS (20, 26).

Despite measurable cell-mediated immune responses to HSV in immunocompetent individuals, the virus can establish a latent infection in neuronal tissue, and frequent recurrences can still occur. Understanding the nature of this HSV-host interaction is made more difficult by the fact that herpesviruses themselves are immunosuppressive (2, 22).

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Indeed, it is not clear whether immunosuppression leads to reactivation of HSV or whether HSV reactivation results in immunosuppression.

In this study, we analyzed the ability of human skin fibroblasts (FB) infected with HSV to be recognized by HSV-specific CTL. FB infected with HSV (HSV-FB) were previously reported to be lysed by $CD8^+$ anti-HSV CTL generated after in vitro exposure of peripheral blood lymphocytes to irradiated FB absorbed with UV-inactivated HSV (30). FB have also been shown to serve as target cells for CTL specific for cytomegalovirus, another member of the herpesvirus family (3, 4). It was thus surprising to find that, in contrast to HSV-infected human Epstein-Barr virus-transformed B-cell lines (LCL), which were readily lysed by autologous anti-HSV CTL, HSV-FB were resistant to lysis by these CTL. We further showed that HSV-FB were resistant to alloantigen-specific CTL-mediated lysis. Although the resistance of HSV-FB to anti-HSV CTL and alloantigen-specific CTL-mediated lysis might be interpreted as resulting from a lack of appropriate MHC-viral epitope expression by the target cells, our findings were reminiscent of those reported by Confer et al. (5). These investigators used sandwich assays to demonstrate that incubation of human natural killer (NK) and lymphokine-activated killer (LAK) cells with a monolayer of HSV-FB or HSV-infected human endothelial cells, but not uninfected FB or endothelial cells, "disarmed" the killers in that they were unable to lyse sensitive target cells.

Here we support and extend the findings of Confer et al. (5) and demonstrate that HSV-FB inactivate human anti-HSV CTL and alloantigen-specific CTL by a contact-dependent mechanism resulting in the inability of CTL to lyse normally sensitive targets. Thus, the resistance of HSV-FB to anti-HSV CTL and alloantigen-specific CTL is not due to an inherent resistance of the target cells; rather, the resistance results from the ability of HSV-FB to inhibit a wide variety of cytotoxic effector-cell activities. The ability of human HSV-FB to inhibit local CTL-mediated lysis may be a mechanism of virus-induced immunosuppression that permits HSV to spread and persist in immunocompetent hosts after primary infection or reactivation of latent HSV.

MATERIALS AND METHODS

Cell lines and viruses. LCL were established by infecting 2.5×10^6 peripheral blood mononuclear cells (PBMC) overnight with 2.5 ml of cell-free supernatant of B95-8 cells. Cells were then pelleted and resuspended in 5 ml of RPMI 1640 medium containing 20% heat-inactivated fetal bovine serum (FBS), 102 U of penicillin G per ml, 10 μg of streptomycin sulfate per ml, 2 mM L-glutamine, 10 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) (all from GIBCO Laboratories, Grand Island, N.Y.), and 1 μg of cyclosporin A (Sandoz Canada, Montreal, Quebec, Canada) per ml in a 25-cm^2 flask. After 7 days, cells were pelleted, resuspended, and maintained in RPMI 1640 medium containing 10% FBS, 102 U of penicillin G per ml, 10 μg of streptomycin sulfate per ml, 2 mM L-glutamine, and 10 mM HEPES (hereafter termed culture medium). Human skin FB were obtained by skin biopsy from the underside of the forearm and were maintained in α minimal essential medium containing 10% FBS, 102 U of penicillin G per ml, 10 μg of streptomycin sulfate per ml, 2 mM L-glutamine, and 10 mM HEPES (hereafter termed FB medium). K562 cells, an NK cell-sensitive human erythroleukemia cell line, were maintained in culture medium.

HSV type 1 (HSV-1) strain F (obtained from P. Spear, University of Chicago) and HSV-2 strain 333 were propagated, and titers were determined on Vero cell monolayers maintained in α minimal essential medium containing 8% FBS. Vaccinia virus strain WR (obtained from the American Type Culture Collection) was grown and titers were determined on CV-1 cells. Human adenovirus type 5 (obtained from F. Graham, McMaster University) was grown in HeLa cells, and titers were determined on 293 cells.

[^{35}S]methionine labeling, immunoprecipitation, and SDS-polyacrylamide gel electrophoresis. FB were left uninfected or were infected for various times with 10 PFU of HSV per cell. In the last 2 h of infection, the cells were washed twice in medium 199 lacking methionine and metabolically labeled with 50 μCi of [^{35}S]methionine (ICN Biomedicals Canada Ltd., St. Laurent, Quebec, Canada) in 1 ml of medium 199 lacking methionine. Cells were then washed twice in phosphate-buffered saline (PBS) and cell lysates were prepared on ice with 1 ml of radioimmunoprecipitation assay (RIPA) buffer (pH 7.2) (50 mM Tris, 0.15 M NaCl, 1% Triton X-100, 1% Na deoxycholate, 0.1% sodium dodecyl sulfate [SDS], 100 KIU of aprotonin [Sigma Chemical Co., St. Louis, Mo.] per ml). HSV-specific proteins were immunoprecipitated from cell extracts with protein A-Sepharose (Pharmacia Chemicals, Dorval, Quebec, Canada) and rabbit anti-HSV-1 immune serum (kindly provided by W. Rawls, McMaster University) for 4 h at 4°C. Immunoprecipitates were washed three times in cold RIPA buffer, 50 μl of SDS sample buffer was added, and samples were heated at 100°C for 5 min. Samples (40 μl) were loaded onto a continuous 10% polyacrylamide gel and electrophoresed at 70 V overnight. Gels were fixed in water-methanol-acetic acid (50:50:7) for 1 h, infused with Enlightning (New England Nuclear Corp., Boston, Mass.) for 15 min., dried, and exposed to Kodak XAR film.

Immunofluorescence. Subconfluent monolayers of FB grown on coverslips were left uninfected or were infected for various times with 10 PFU of HSV per cell. Cells on coverslips were fixed in cold acetone for 10 min and allowed to air dry before the coverslips were mounted onto microscope slides. Slides were washed twice with PBS containing 0.1% bovine serum albumin (PBS-BSA), and 40 μl of a 1:8 dilution of rabbit anti-HSV-1 immune serum was added. Slides were incubated at 37°C for 30 min and then washed six times with PBS-BSA. Slides were then incubated for 30 min at 37°C with 40 μl of a 1:20 dilution of fluorescein isothiocyanate-goat anti-rabbit immunoglobulin G. Slides were washed six times with PBS-BSA, 1 drop of glycerol-based aqueous mounting solution was added, and slides were covered with a coverslip. Fluorescence was viewed with a Leitz inverted fluorescence microscope.

Generation of effector cells. Anti-HSV CTL were prepared as described previously (31) with modifications. Briefly, PBMC were isolated from HSV-seropositive donors by using Ficoll-Paque (Pharmacia) and resuspended at 10^6/ml in culture medium containing 0.02 mM 2-mercaptoethanol (Sigma Chemical Co., St. Louis, Mo.). Ten million cells were cultured at 37°C in 5% CO_2 in 25-cm^2 upright flasks with 10^6 PFU of HSV. After 7 days, cells were harvested as primary effectors.

Alloantigen-specific CTL were generated as above by replacing HSV with 10^6 irradiated (2,000 rads) allogeneic PBMC as stimulators.

Cell-mediated cytotoxicity assays. Standard chromium release assays were used to assess CTL-mediated cytotoxicity. LCL and FB, used as target cells, were left uninfected or

were infected for various times with 10 PFU of HSV per cell. In the final 1.5 h of infection, cells were labeled with 200 μCi of $Na_2{}^{51}CrO_4$ (New England Nuclear Corp.), washed, and counted. Target cells (1×10^4 to 2×10^4) were added to effectors at various effector/target cell ratios in 96-well flat-bottomed microtiter plates (Nunclon, Roskilde, Denmark) and incubated for 5 h at 37°C in 5% CO_2. Target cells were also incubated in culture medium alone (spontaneous release) or with 1 N HCl (maximal release). After 5 h of incubation, 100 μl of supernatant was removed from each well and its radioactivity was counted in a gamma counter. The percent specific ^{51}Cr release was calculated as follows: [(cpm experimental release − cpm spontaneous release)/(cpm maximal release − cpm spontaneous release)] × 100, where cpm is counts per minute. The spontaneous release was always less than 30% of the maximal release. In some cases, OKT3, a monoclonal antibody specific for CD3 which blocks killing by $CD3^+$ cells, was added to wells before the addition of target cells.

In some experiments, cytotoxicity was determined by a sandwich assay as described by Confer et al. (5) with slight modifications. Briefly, uninfected FB and FB infected for 2, 4 or 20 h with 10 PFU of HSV were trypsinized, and 10^4 cells per well were added to 96-well flat-bottomed plates. FB were allowed to adhere and recover for 1 to 2 h. Afterward, effectors were added to the FB for 2, 4, or 6 h before the addition of ^{51}Cr-labeled target cells in a ^{51}Cr release assay.

The ability of effector cells to lyse HSV-FB via antibody-dependent cellular cytotoxicity (ADCC) was also determined. Briefly, human PBMC were isolated and tested for cytotoxicity against ^{51}Cr-labeled autologous uninfected FB or HSV-FB in the absence or presence of rabbit anti-HSV immune serum (1:80 dilution), which was added at the start of a ^{51}Cr release assay.

Depletion of T-cell and NK-cell subsets. For elimination of $CD4^+$, $CD8^+$, $CD56^+$, or $CD16^+$ cells, effectors were resuspended at 3×10^6/ml in PBS-BSA containing anti-leu3a (anti-CD4), anti-leu2b (anti-CD8), anti-leu19 (anti-CD56), or anti-leu11b (anti-CD16) (Becton Dickinson Immunocytometry Systems, Mountain View, Calif.) at 1:100 and incubated for 30 min at 4°C. Cells were washed three times with PBS-BSA and resuspended at 5×10^6/ml. DYNABEADS M-450 coated with goat anti-mouse immunoglobulin G (DYNAL Inc., Great Neck, N.Y.) were added to cells treated with anti-leu3a, anti-leu2b, and anti-leu19 at a bead/cell ratio of 10:1. The cells and beads were incubated for 1 h at 4°C with continuous rocking. The beads with attached cells were then magnetically removed, and the remaining cells were resuspended in culture medium and used as effectors. Two milliliters of a 1:10 dilution of Low-Tox-H rabbit complement (Cedarlane, Hornby, Ontario, Canada) was added to cells treated with anti-leu11b and incubated for 1 h at 37°C. Cells were then washed three times with PBS-BSA, resuspended in culture medium, and used as effectors.

Role of soluble FB factors in CTL inhibition. To determine whether soluble factors were involved in inhibition of CTL activity, we obtained conditioned supernatant from uninfected FB or from FB infected overnight with 10 PFU of HSV and passed through a 0.22-μm-pore-size filter. Conditioned supernatant (250 μl) was then added to effectors for 4 h before the addition of ^{51}Cr-labeled targets. Additionally, Transwells (Costar, Cambridge, Mass.) were used to maximize exposure of effector cells to FB supernatants without direct contact. FB (5×10^4 per well) were grown in a 24-well cluster plate (Costar). Cells were left uninfected or were infected with 10 PFU of HSV per cell in 0.5 ml of α-minimal essential medium with 5% FBS. After 30 min of absorption of HSV, the inoculum was removed and 1 ml of FB media was added per well. A 6.5-mm-diameter tissue culture-treated Transwell with a 0.4-μm pore size (Costar) was placed in each well, and 200 μl of FB medium was added to the Transwell. Culture supernatant was allowed to equilibrate overnight through the porous polycarbonate filter. Alloantigen-specific CTL (200 μl) were added to the Transwell (1×10^6 to 2×10^6 effectors per Transwell) for 4 h; this was followed by their removal and addition to ^{51}Cr-labeled target cells.

TABLE 1. Resistance of HSV-FB to anti-HSV CTL-mediated lysis

Target[a]	Cell population depleted[b]		
	CD16 + CD56	CD16 + CD56 + CD4	CD16 + CD56 + CD8
auto LCL	5	5	3
auto HSV-LCL	35	16	20
allo HSV-LCL	7	6	3
auto FB	4	3	1
auto HSV-FB	6	2	3

[a] Target cells were uninfected autologous (auto) LCL or FB, or auto LCL, auto FB, or allogeneic (allo) LCL infected for 6 h with 10 PFU of HSV per cell.

[b] PBMC from an HSV-seropositive donor were stimulated with HSV for 7 days and were depleted of NK cells ($CD16^+$ and $CD56^+$ cells) and $CD4^+$ or $CD8^+$ cells just before use in a ^{51}Cr release assay. Percent specific ^{51}Cr release is shown at an effector/target cell ratio of 50:1; effector cells were not readjusted after $CD4^+$ or $CD8^+$ cells were depleted.

Statistical analysis. Results were analyzed by a Student's two-tailed *t* test with MINITAB statistical software. A *P* value of <0.05 was taken to be significant.

RESULTS

Resistance of HSV-FB to lysis by human anti-HSV CTL. HSV-FB were tested for sensitivity to lysis by human anti-HSV CTL. Human anti-HSV CTL, generated from PBMC of HSV-seropositive donors and depleted of $CD16^+$ and $CD56^+$ NK cells, were tested for lytic activity against autologous LCL and HSV-FB. Although anti-HSV CTL clearly lysed LCL infected for 6 h with HSV, no lysis of HSV-FB was detected (Table 1). Anti-HSV CTL were virus specific and MHC restricted since neither uninfected autologous LCL nor HSV-infected allogeneic LCL were lysed (Table 1). Depletion of either $CD4^+$ or $CD8^+$ cells from the bulk population of effectors reduced, but did not eliminate, the killing of HSV-infected autologous LCL (Table 1). These results demonstrate that both $CD4^+$ and $CD8^+$ anti-HSV CTL were present in the effector population. Although Table 1 displays the lytic activity of anti-HSV CTL from a single HSV-seropositive donor, anti-HSV CTL generated from all donors tested did not lyse autologous HSV-FB (data not shown). Further, human FB infected for 18 and 24 h with HSV were also fully resistant to lysis by autologous anti-HSV CTL (data not shown). Thus, our results demonstrate that human FB infected with HSV are resistant to lysis by autologous anti-HSV CTL which are capable of lysing virus-infected LCL. Our results do not support those of Yasukawa et al. (30), who showed that human FB infected overnight with HSV were sensitive to $CD8^+$ human anti-HSV CTL.

Expression of HSV glycoproteins in HSV-FB. To ensure

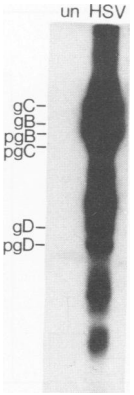

FIG. 1. Immunoprecipitation of HSV glycoproteins in HSV-FB. Human FB were left uninfected (un) or were infected for 20 h with 10 PFU of HSV per cell (HSV). HSV proteins were immunoprecipitated with rabbit anti-HSV-1 immune serum and protein A-Sepharose. The immature forms of glycoproteins B (gB), C (gC), and D (gD) are indicated by pgB, pgC, and pgD, respectively.

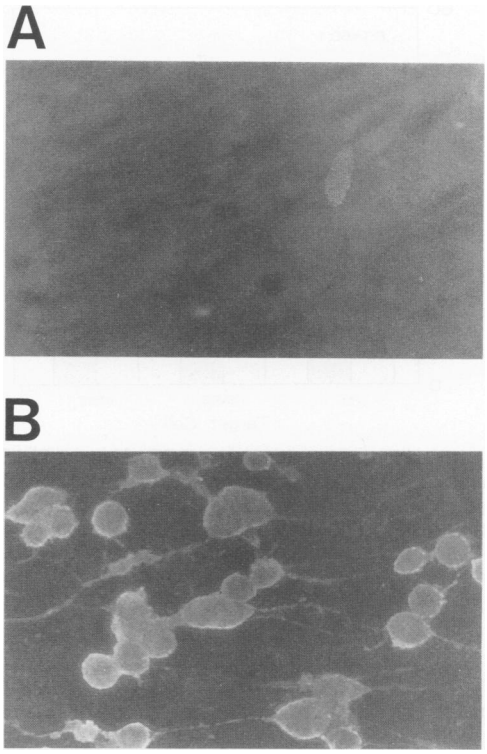

FIG. 2. Expression of HSV proteins in human FB. Human FB grown on coverslips were left uninfected (A) or were infected for 20 h with 10 PFU of HSV per cell (B) and fixed in cold acetone. Coverslips were incubated with rabbit anti-HSV-1 immune serum and then incubated with fluorescein isothiocyanate-labeled goat anti-rabbit immunoglobulin G. Cells incubated with fluorescein isothiocyanate-labeled goat anti-rabbit immunoglobulin G alone displayed no detectable fluorescence.

that the resistance of HSV-FB to $CD8^+$ anti-HSV CTL was not due to lack of infectability or nonproduction of HSV proteins, we determined the expression of HSV glycoproteins in HSV-FB. Figure 1 demonstrates that FB infected with HSV express abundant levels of HSV-specific glycoproteins. To determine the proportion of FB infectible with HSV, we performed immunofluorescence on monolayers of human FB infected with HSV. Greater than 90% of FB stained positively for HSV-specific proteins, whereas no positively staining cells were observed in a monolayer of uninfected FB (Fig. 2). These results demonstrate that HSV infects and expresses abundant levels of viral proteins in human FB and thus that resistance of HSV-FB to anti-HSV CTL was not due to a lack of infectivity or viral protein production.

Sensitivity of HSV-infected human FB to ADCC. Although HSV-FB were not lysed by human anti-HSV CTL, HSV-FB infected for 6 h with HSV were sensitive to ADCC (Fig. 3). In contrast to the NK cell-sensitive K562 cell line, uninfected FB and HSV-FB were weakly sensitive to lysis by fresh PBMC. Addition of rabbit anti-HSV immune serum to the FB and PBMC resulted in a marked increase in lysis of HSV-FB but not uninfected FB (Fig. 3). These results demonstrate that infection of FB with HSV renders them sensitive to the lytic machinery triggered through the Fc receptor on human NK cells and that HSV-FB are not resistant to all forms of cell-mediated lysis.

Resistance of HSV-FB to alloantigen-specific CTL. Human alloantigen-specific CTL, depleted of $CD16^+$ and $CD56^+$ cells, lysed allogeneic LCL and uninfected FB (Fig. 4). However, the lysis by alloantigen-specific CTL of FB infected for 4 h with HSV was reduced relative to the lysis of uninfected FB, and infection for 20 h rendered the cells fully resistant to lysis (Fig. 4). Thus, human FB infected with HSV demonstrated a time-dependent resistance to lysis by alloantigen-specific CTL. Alloantigen-specific CTL were specific for allogeneic cells since neither autologous LCL nor FB were killed (Fig. 4). Further, depletion of $CD16^+$ and $CD56^+$ NK cells was successful as demonstrated by the low level of lysis of K562 cells (Fig. 4). Therefore, although sensitive to ADCC, human FB infected with HSV for over 4 h were fully resistant to both autologous human anti-HSV CTL and alloantigen-specific CTL.

Inactivation of CTL by HSV-FB. Although one may interpret the resistance of HSV-FB to anti-HSV CTL- and alloantigen-specific CTL-mediated lysis as a result of lack of MHC expression, Confer et al. (5) demonstrated that incubation of human NK and LAK cells with a monolayer of human FB or endothelial cells infected with HSV, but not uninfected FB or endothelial cells, disarmed the killers in that they were unable to lyse sensitive target cells. To determine whether a similar phenomenon was occurring with human CTL, we conducted sandwich assays. For these assays, anti-HSV CTL were incubated for 2 h with human FB that had previously been infected with HSV for 20 h or left uninfected. Afterwards, chromium-labeled target cells were added and cytotoxicity was measured. Results shown in Fig. 5 demonstrate that anti-HSV CTL incubated on uninfected FB clearly lysed HSV-infected autologous LCL. In contrast, incubation of anti-HSV CTL on HSV-FB rendered these CTL incapable of lysing HSV-infected LCL (Fig. 5). The inhibition of anti-HSV CTL lytic activity by incubation with HSV-FB ranged from 61% at an effector/target cell ratio of 50:1 to 81% at an effector/target cell ratio of 25:1 relative to the lysis mediated by anti-HSV CTL incubated on uninfected FB ($P < 0.05$).

Human alloantigen-specific CTL were also sensitive to the inhibitory effects of HSV-FB. Incubation of alloantigen-specific CTL with HSV-FB inhibited the lysis of sensitive targets by these CTL in a time-dependent manner, i.e., the

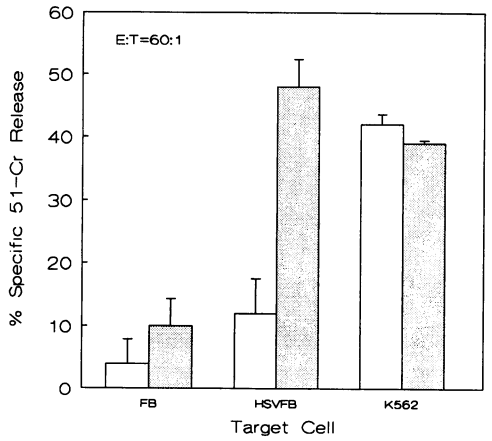

FIG. 3. HSV-FB are sensitive to ADCC. Human PBMC were isolated and tested for lytic activity against ^{51}Cr-labeled FB (FB), FB infected for 6 h with 10 PFU of HSV per cell (HSVFB), or K562 cells in the absence (fresh PBMC [□]) or presence of rabbit anti-HSV-1 immune serum (fresh PBMC plus anti-HSV [▩]). Results shown are with an effector/target cell ratio of 60:1. Error bars represent standard deviation from the mean of triplicate wells.

longer the incubation of effectors with HSV-FB, the greater the inhibition (Fig. 6). The inhibition of alloantigen-specific CTL by HSV-FB relative to incubation on uninfected FB was 38, 58, and 79% at 2, 4, and 6 h, respectively ($P < 0.05$). Indeed, after 4 h of contact between alloantigen-specific CTL and HSV-FB, lysis of susceptible allogeneic targets was reduced to background levels seen with effectors treated with anti-CD3 (Fig. 6). The specificity of the alloantigen-specific CTL was shown by the marked lysis of allogeneic targets and the inability to lyse autologous labeled target cells (Fig. 6). These data suggest that the resistance of HSV-FB to lysis by human anti-HSV CTL and alloantigen-specific CTL was due to the ability of HSV-FB to inhibit the lytic capacity of CTL rather than an intrinsic resistance of HSV-FB to CTL-mediated lysis.

To further characterize the inhibition of CTL activity

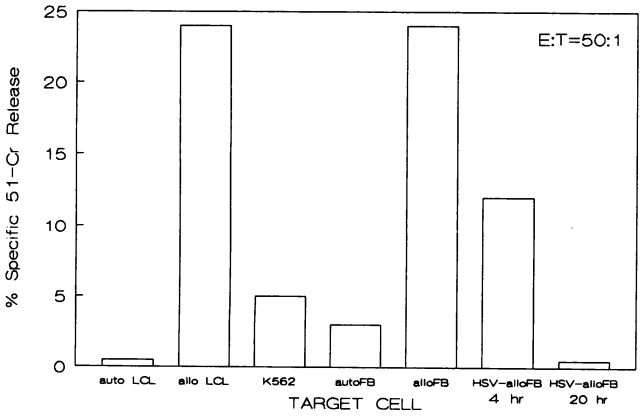

FIG. 4. Resistance of HSV-FB to alloantigen-specific CTL-mediated lysis increases with time. Alloantigen-specific CTL were generated, depleted of CD16$^+$ and CD56$^+$ cells by using anti-leu19 and anti-leu11b, respectively, and tested for lytic activity against ^{51}Cr-labeled K562 cells, autologous or allogeneic LCL, autologous or allogeneic FB, or allogeneic FB infected for 4 or 20 h with 10 PFU of HSV. Results shown are with an effector/target cell ratio of 50:1.

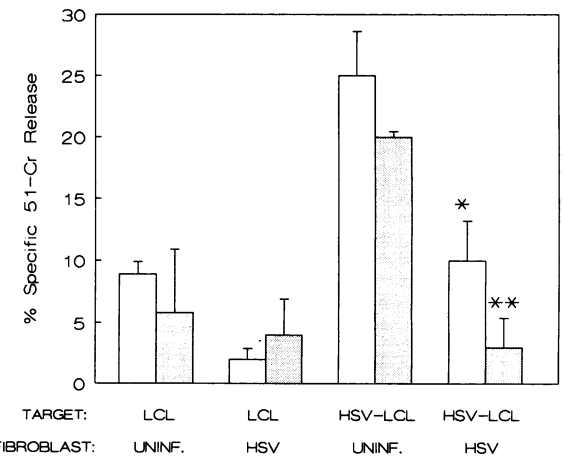

FIG. 5. Anti-HSV CTL are inhibited from lysis of sensitive targets when incubated on HSV-FB. Human primary anti-HSV CTL were generated and incubated for 2 h on uninfected skin FB or FB previously infected with 10 PFU of HSV for 20 h. After incubation, ^{51}Cr-labeled autologous uninfected LCL or LCL infected overnight with 10 PFU of HSV (HSV-LCL) were added in a 5-h ^{51}Cr release assay. Error bars represent standard deviation from the mean of triplicate wells. *, 61% inhibition of lysis of effectors at an effector/target cell ratio of 50:1 (□) compared with effectors incubated on uninfected FB ($P < 0.05$). **, 88% inhibition of lysis of effectors at an effector/target cell ratio of 25:1 (▩) compared with effectors incubated on uninfected FB ($P < 0.05$).

mediated by HSV-FB, we determined the time of infection of human FB required for inhibition of lytic activity to be observed. Human FB were infected for 2, 4, or 20 h with HSV before a 2-h exposure to alloantigen-specific CTL.

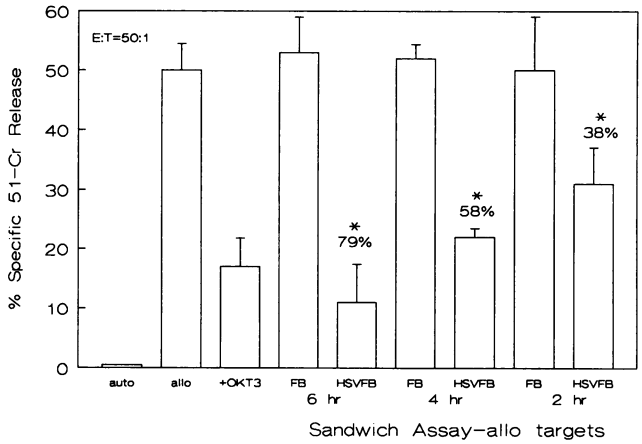

FIG. 6. Inhibition of lysis by alloantigen-specific CTL increases with increasing time of incubation of effectors with HSV-FB. Human alloantigen-specific CTL were generated and left untreated and tested for lytic activity against ^{51}Cr-labeled autologous LCL (auto) or allogeneic LCL (allo). Anti-CD3 (OKT3) was added to an additional group of effectors and allogeneic LCL (+OKT3). Effectors were also incubated on monolayers of FB or HSV-FB for 2, 4, or 6 h in a sandwich assay. After incubation, ^{51}Cr-labeled allogeneic LCL were added. Results shown are with an effector/target cell ratio of 50:1; error bars represent the standard deviation from the mean of quadruplicate wells. *, inhibition of alloantigen-specific CTL at 6, 4, and 2 h is 79, 58, and 38%, respectively, compared with lysis by alloantigen-specific CTL incubated on uninfected FB for the same time ($P < 0.05$).

TABLE 2. Inhibition of alloantigen-specific CTL lysis by FB infected for various times with HSV

Cell[a]	Expt 1[b]	% Inhibition[c]	Expt 2[b]	% Inhibition[c]
auto LCL	5		0	
allo LCL	70		43	
+OKT3	16		11	
Sandwich assay[d]				
unFB	68		41	
HSV-FB				
2 h	41	40	15	63
4 h	38	44	15	63
20 h	48	29	28	32

[a] Target cells were allogeneic (allo) LCL except for autologous (auto) LCL control targets.

[b] Results shown are percent specific ^{51}Cr release at an effector/target cell ratio of 60:1 for experiments 1 and 2.

[c] Percent inhibition of alloantigen-specific CTL lysis by HSV-FB compared with lysis of alloantigen-specific CTL incubated on uninfected FB (unFB).

[d] Alloantigen-specific CTL were incubated for 2 h on uninfected FB or FB previously infected for 2, 4, or 20 h with HSV before the addition of ^{51}Cr-labeled allo LCL.

TABLE 3. Inhibition of alloantigen-specific CTL by HSV-FB is not mediated by a soluble factor[a]

Expt	auto[b]	allo	OKT3	Sandwich		Supernatant		Transwell	
				un	HSV	un	HSV	un	HSV
1	4	48	3	54	**23**[c]	54	54	—[d]	—
2	1	16	1	22	**9**	21	21	—	—
3	0	48	14	43	**13**	—	—	44	35
4	8	40	10	43	**10**	—	—	37	34

[a] To determine whether a soluble product was responsible for the inhibition of CTL activity, we determined lysis of ^{51}Cr-labeled allogeneic LCL after incubation of effector alloantigen-specific CTL for 4 h in a sandwich assay on uninfected FB (un) or FB infected for 20 h with HSV (HSV). Lysis of targets in a sandwich assay was compared with lysis mediated by alloantigen-specific CTL incubated for 4 h with filtered supernatant collected from uninfected FB or HSV-FB (experiments 1 and 2) or compared with lysis mediated by alloantigen-specific CTL incubated for 4 h in a 0.4-μm-pore-size Transwell exposed to uninfected FB or HSV-FB (experiments 3 and 4). Results shown are percent specific ^{51}Cr release at an effector/target cell ratio of 50:1 except for experiment 1, which was 60:1.

[b] Target cells were allogeneic (allo) LCL except for autologous (auto) control LCL targets.

[c] Boldface numbers are significantly different from cells incubated on uninfected FB ($P < 0.05$).

[d] —, not done.

Results demonstrated that by 2 h postinfection, human FB were able to profoundly inhibit the cytotoxic activity of alloantigen-specific CTL (Table 2). Indeed, FB infected for 2 and 4 h inhibited CTL activity to a greater extent than FB infected for 20 h (Table 2). These results are distinct from those reported by Confer et al. (5), who demonstrated that the degree of inhibition of NK- and LAK-cell lysis was dependent on the duration of HSV infection and was only manifest after 18 h of infection.

To determine whether the inhibition of CTL activity was specific for HSVs, we incubated alloantigen-specific CTL in sandwich assays with FB infected with HSV-1, HSV-2, vaccinia virus, or human adenovirus type 5. Results shown in Fig. 7 demonstrate that incubation of alloantigen-specific CTL for 4 h with FB infected with HSV-1 or HSV-2 significantly inhibited cytotoxic activity. Indeed, lysis was inhibited to levels observed after incubation of effector cells with anti-CD3. In contrast, incubation of alloantigen-specific CTL on FB left uninfected or infected with vaccinia virus or adenovirus type 5 did not inhibit lysis of relevant alloantigenic LCL target cells (Fig. 7). Thus, inactivation of alloantigen-specific CTL appears to be specific for HSV-FB.

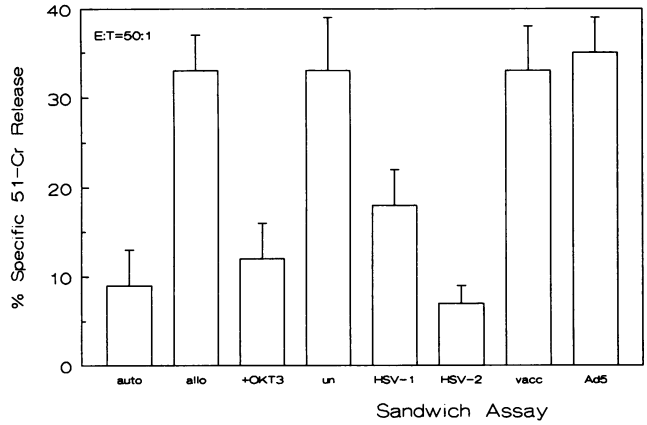

FIG. 7. Alloantigen-specific CTL are inactivated by HSV-FB but not FB infected with vaccinia virus or adenovirus. Human alloantigen-specific CTL were generated and left untreated and tested for lytic activity against ^{51}Cr-labeled autologous LCL (auto) or allogeneic LCL (allo). Anti-CD3 (OKT3) was added to an additional group of effectors and allogeneic LCL (+OKT3). Effectors were also incubated on monolayers of uninfected FB (un) or on FB infected for 20 h with HSV-1, HSV-2, vaccinia virus (vacc), or human adenovirus type 5 (Ad5) for 4 h in a sandwich assay. After incubation, ^{51}Cr-labeled allogeneic LCL were added. Results shown are with an effector/target cell ratio of 50:1; error bars represent the standard deviation from the mean of triplicate wells.

Inhibition of alloantigen-specific CTL is not mediated by a soluble factor. To determine whether a soluble product secreted from HSV-FB mediated the inhibitory effect on human CTL, we obtained cell-free supernatant from monolayers of uninfected FB or FB that were infected with HSV overnight. Supernatant was added to alloantigen-specific CTL for 4 h before the addition of ^{51}Cr-labeled sensitive target cells (allogeneic LCL). Results (Table 3, experiments 1 and 2) demonstrated that human alloantigen-specific CTL were not inhibited by a product present in the supernatant from HSV-FB. Indeed, lysis of allogeneic LCL was equivalent whether alloantigen-specific CTL were incubated with supernatant from uninfected FB or HSV-FB. In contrast, alloantigen-specific CTL-mediated lysis was significantly inhibited when the effector cells were incubated on HSV-FB in a sandwich assay (Table 3, experiments 1 and 2). Similar results were obtained when alloantigen-specific CTL were incubated for 2 or 6 h with supernatants from HSV-FB (data not shown).

To maximize exposure of effector CTL to FB supernatants, we used Transwell culture dishes. Each well of these culture dishes has a removable top chamber that is separated from the bottom chamber by a 0.4-μm-pore-size filter. Incubation of alloantigen-specific CTL in Transwell culture dishes that permitted exposure to FB supernatant from uninfected FB and HSV-FB without direct contact did not result in inhibition of cytotoxic activity (Table 3, experiments 3 and 4). In contrast, alloantigen-specific CTL were inhibited in sandwich assays when contact was permitted with HSV-FB but not with uninfected FB (Table 3). For both of these assays, the effectors were shown to be $CD3^+$ alloantigen-specific CTL since the effectors did not lyse

autologous labeled LCL and since lysis was inhibited in the presence of anti-CD3 antibody (Table 3). Although the data in Table 3 were generated with FB that had been infected overnight, FB infected with HSV for 2 or 4 h also did not inhibit alloantigen-specific CTL activity in a Transwell system (data not shown). Thus, the inhibitory effect mediated by HSV-FB on alloantigen-specific CTL does not appear to be mediated by a soluble factor but rather requires cell-to-cell contact.

DISCUSSION

In this study, we analyzed the recognition and lysis of HSV-FB by human anti-HSV CTL and alloantigen-specific CTL. In contrast to LCL, which were lysed by HLA-restricted anti-HSV CTL, at no time postinfection were HSV-FB lysed by human anti-HSV CTL. This resistance to antiviral CTL-mediated lysis was not due to a lack of infectivity of the target cells, since HSV-FB clearly expressed abundant amounts of HSV-specific proteins. Infection of FB with HSV also resulted in a time-dependent resistance to alloantigen-specific CTL-mediated lysis. Alloantigen-specific CTL lysis of FB infected for 4 h with HSV was reduced relative to the lysis of uninfected allogeneic FB, and infection for 20 h rendered the cells fully resistant to lysis. The apparent resistance of HSV-FB to human CTL was reminiscent of studies reported by Confer et al. (5), who demonstrated that human FB infected overnight with HSV were not lysed by human NK or LAK cells. The mechanism of resistance of HSV-FB to human NK- and LAK-cell lysis was shown to be due to disarming of the killer cells by direct contact with HSV-FB (5). Here we extend the results of Confer et al. (5) and demonstrate that incubation of anti-HSV CTL or alloantigen-specific CTL with HSV-FB for 2 to 6 h rendered the CTL incapable of lysing their normally sensitive target cells. Indeed, by only 2 h postinfection with HSV, human FB were able to profoundly inhibit the cytotoxic activity of alloantigen-specific CTL. This effect appeared to be HSV specific since infection of FB with HSV-1 or HSV-2 resulted in CTL inactivation, whereas infection of FB with adenovirus or vaccinia virus did not diminish CTL activity. Last, the inhibition of CTL by HSV-FB is not mediated by a soluble factor, but rather requires cell-to-cell contact. Thus, HSV-FB are not inherently resistant to lysis by anti-HSV CTL or alloantigen-specific CTL, but rather contact of CTL with HSV-FB resulted in inactivation of the CTL.

Surprisingly, the first evidence that HLA-restricted HSV-specific CTL-mediated cytotoxicity could be demonstrated in humans was shown using skin FB cultures from HLA-typed individuals (25). Subsequently, though, studies of human anti-HSV CTL have relied heavily on the use of autologous LCL as target cells (30–35). Clearly, this was due to the need for HLA-matched target cells and the ease with which LCL can be generated from the peripheral blood of individuals. Since LCL express both class I and II HLA molecules, it was shown that humans have two distinct populations of anti-HSV CTL (24, 30, 32). One is $CD4^-$ $CD8^+$ and restricted by HLA class I, and the other is $CD4^+$ $CD8^-$ and restricted by HLA class II. Activation of both $CD4^+$ and $CD8^+$ CTL by stimulation with UV-inactivated HSV was confirmed by using HSV-infected human monocytes and macrophages as targets (27). Although two subsets of anti-HSV CTL exist in humans, their specificity and role in HSV infections are not well understood.

Studies performed by Yasukawa et al. (30) demonstrated that HLA class II-restricted $CD4^+$ CTL were mainly induced by stimulating peripheral blood lymphocytes with UV-inactivated cell-free HSV antigen, whereas HLA class I-restricted $CD8^+$ CTL were induced by culturing peripheral blood lymphocytes with autologous FB that were absorbed with UV-inactivated HSV. Further, in contrast to our findings, they demonstrated that the $CD8^+$ CTL generated after stimulation with HSV-pulsed autologous FB were able to kill HSV-infected autologous FB (30). Similar results demonstrating the ability of virus-infected human FB to stimulate class I HLA-restricted CTL were reported for human cytomegalovirus (4) and varicella-zoster virus (9).

We were unable to confirm the findings of Yasukawa et al. (30). Indeed, we found that HSV-FB were resistant to $CD8^+$ anti-HSV CTL. This resistance was not due to a lack of infectivity or viral protein production. Human FB infected with HSV expressed abundant levels of HSV-specific proteins as demonstrated by immunoprecipitation, immunofluorescence, and their susceptibility to ADCC in the presence of anti-HSV serum. Although human FB were infectible with HSV, expression of viral proteins does not guarantee the susceptibility of target cells to CTL-mediated lysis. We are now well aware that CTL recognize processed fragments of viral proteins selectively bound by MHC molecules (28). For appropriate antigen presentation to occur, viral proteins must be properly degraded to peptides in the cytoplasm and transported into the endoplasmic reticulum and selectively interact with a folding MHC molecule. Appropriately folded molecules with their bound peptides are then transported to the cell surface for recognition by appropriate CTL. Although we did not pursue problems of HSV antigen processing or presentation in infected human FB, our results indicate that the resistance of HSV-FB to human CTL is a consequence of inactivation of cytotoxic activity by HSV-FB.

Initial studies by Fitzgerald et al. (7) demonstrated that acutely infected human FB were sensitive to human NK-cell activity. Subsequently, though, they found that FB infected for longer periods (20 h) were markedly less susceptible to NK-cell lysis (8). These observations were extended by Confer et al. (5), who demonstrated that human FB and endothelial cells became progressively less susceptible to NK and LAK cell-mediated lysis with duration of HSV infection. Using sandwich assays in which NK or LAK cells were placed atop a monolayer of HSV-FB for variable times before the addition of labeled, susceptible target cells, they demonstrated that human FB infected with HSV potently inhibited the lytic activity of NK and LAK cells.

Our results further extend those of Confer et al. (5) and clearly demonstrate that human FB infected with HSV are also able to potently inhibit the lytic activity of both MHC-restricted $CD3^+$ anti-HSV CTL and alloantigen-specific CTL. Indeed, exposure of anti-HSV CTL to HSV-FB resulted in the inhibition of both $CD4^+$ and $CD8^+$ HLA-restricted anti-HSV CTL since lysis of HSV-infected LCL target cells was abrogated. It is likely that cytolytic cells with any specificity will be inactivated by HSV-FB. Interestingly, the kinetics of infection of human FB needed to inhibit alloantigen-specific CTL differed from that observed with NK and LAK cells. While at least 8 h of FB infection were required to observe inhibition of NK- and LAK-cell lysis which increased with duration of infection, human FB infected for only 2 hours were able to profoundly inhibit alloantigen-specific CTL. Indeed, FB infected for 2 and 4 h inhibited CTL activity to a greater extent than FB infected for 20 h.

Confer et al. (5) referred to the ability of HSV-FB to inhibit killer lymphocytes as "disarming." We have elected not to use this term until we better understand the mechanism of the inhibition. Disarming implies a loss of cytolytic machinery. In preliminary experiments (18), we have found that CTL that were incubated with HSV-FB could subsequently mediate antibody-redirected cytolysis of target cells bearing Fc receptors and incubated with anti-CD3 antibodies. These results imply that CTL still have intact cytolytic machinery but are inhibited from killing susceptible targets. Further, we have found that CTL are inactivated for at least 20 h, but we do not know the duration of inhibition nor whether the effect is transient or results in long-term ablation of lytic activity.

Soluble products released from certain cell types have been shown to inhibit the proliferation of T cells and the lytic phase of human CTL. Transforming growth factor β, produced from an HSV-2-induced murine tumor cell line, was shown to suppress proliferative responses in a mixed lymphocyte reaction with human PBMC (19). Additionally, a 20- to 30-kDa molecule released from $CD8^+$ $CD57^+$ lymphocytes obtained from AIDS patients has been shown to inhibit the effector phase of MHC-restricted CTL (23). Recently, it was also shown that supernatants generated by incubating human PBMC with HSV-FB contain tumor necrosis factor (13). In the present study, the filtered supernatant collected from HSV-FB did not mediate inhibition of alloantigen-specific CTL. Further, incubation of CTL in Transwell dishes that permitted exposure to FB supernatant from HSV-FB without direct contact did not result in inhibition of cytotoxic activity. Conditioned medium from HSV-FB was also shown to have no effect on the lytic capability of NK or LAK cells (5). Thus, the inhibitory effect mediated by HSV-FB is not mediated by a soluble factor, but rather requires direct cell-to-cell contact.

Since direct contact with HSV-FB is required for the inhibition of cell-mediated cytotoxicity, it is natural to suspect that a surface-expressed molecule on infected cells is responsible. Confer et al. (5) demonstrated that HSV-FB incubated for 18 h in the presence of tunicamycin, an inhibitor of N-linked glycosylation, lost the capacity to inhibit NK cell-mediated cytolysis. They interpreted their results as suggesting that surface expression of HSV glycoproteins was involved in suppressing the cytotoxic cells. We are currently investigating the role of viral glycoproteins in mediating the inhibitory ability of HSV-FB on CTL-mediated lysis.

Hommel-Berrey et al. (10) have described a contact-dependent mechanism of CTL inactivation by an Epstein-Barr virus-transformed cell line, PAMO, and suggested that it is due to the modulation of a key membrane molecule (e.g., T-cell receptor/CD3 complex, CD4 or CD8 and class I MHC) and the lack of certain secondary messengers involved in signal transduction (10). Taken together with our results and those of Confer et al. (5), these observations imply the existence of a signal transduction pathway in all cytolytic cells whose activation inhibits lytic activity.

The inactivation of human anti-HSV CTL may have important implications in HSV infections. Immunocompromised patients with defects in cell-mediated immunity experience more severe and extensive HSV infections and reactivations than those with deficits in humoral immunity (6). During normal HSV reactivations, suppression of certain immune responses has been detected. For example, immediately after the onset of an HSV-1 lesion, the in vitro production of interleukin-2 and alpha interferon from PBMC stimulated with HSV-1 antigen was suppressed relative to the production of these cytokines from PBMC isolated during the convalescent phase (4 to 14 days after lesion onset) (11). Similarly, cell-mediated cytotoxicity, most likely NK-cell lysis, was also significantly lower in patients in the recrudescent phase relative to the convalescent phase (11). Preliminary data from our laboratory demonstrate that in two donors with frequent HSV recurrences, HSV-specific CTL were undetectable 0 to 5 days before the onset of a lesion, whereas 7 days after the onset, anti-HSV CTL could be measured (18). Taken together, these findings suggest that suppression of CTL activity and other immune parameters is coincident with the onset of an HSV lesion, implying that the virus itself is responsible for a transient immunosuppression leading to viral reactivation. Possibly, infection of FB and endothelial cells with HSV may result in a transient local suppression of cell-mediated immune responses which allows the virus to spread, form a lesion, and infect other ganglia. This could account for the recurrent episodes and persistence of HSV in immunocompetent individuals.

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REFERENCES

1. **Aebischer, T., D. Moskophidis, U. H. Rohrer, R. M. Zinkernagel, and H. Hengartner.** 1991. *In vitro* selection of lymphocytic choriomeningitis virus escape mutants by cytotoxic T lymphocytes. Proc. Natl. Acad. Sci. USA **88**:11047–11051.
2. **Banks, T. A., and B. T. Rouse.** 1992. Herpesviruses-immune escape artists? Cl. Infect. Dis. **14**:933–941.
3. **Borysiewicz, L. K., J. K. Hickling, S. Graham, J. Sinclair, M. P. Cranage, G. L. Smith, and J. G. P. Sissons.** 1988. Human cytomegalovirus-specific cytotoxic T cells: relative frequency of stage-specific CTL recognizing the 72 kD immediate early protein and glycoprotein B expressed by recombinant vaccinia viruses. J. Exp. Med. **168**:919–931.
4. **Borysiewicz, L. K., S. Morris, J. D. Page, and J. G. P. Sissons.** 1983. Human cytomegalovirus-specific cytotoxic T lymphocytes: requirements for *in vitro* generation and specificity. Eur. J. Immunol. **13**:804–809.
5. **Confer, D. L., G. M. Vercellotti, D. Kotasek, J. L. Goodman, A. Ochoa, and H. S. Jacob.** 1990. Herpes simplex virus-infected cells disarm killer lymphocytes. Proc. Natl. Acad. Sci. USA **87**:3609–3613.
6. **Corey, L., and P. G. Spear.** 1986. Infections with herpes simplex viruses. N. Engl. J. Med. **314**:686–691.
7. **Fitzgerald, P. A., R. Evans, D. Kirkpatrick, and C. Lopez.** 1983. Heterogeneity of human NK cells: comparison of effectors that lyse HSV-1-infected fibroblasts and K562 erythroleukemia targets. J. Immunol. **130**:1663–1667.
8. **Fitzgerald-Bocarsly, P., D. M. Howell, L. Pettera, S. Tehrani, and C. Lopez.** 1991. Immediate-early gene expression is sufficient for induction of natural killer cell-mediated lysis of herpes simplex virus type 1-infected fibroblasts. J. Virol. **65**:3151–3160.
9. **Hickling, J. K., L. K. Borysiewicz, and J. G. P. Sissons.** 1987. Varicella-zoster virus-specific cytotoxic T lymphocytes (Tc): detection and frequency analysis of HLA class I-restricted Tc in human peripheral blood. J. Virol. **61**:3463–3469.
10. **Hommel-Berrey, G. A., A. M. Shenoy, and Z. Brahmi.** 1991. Receptor modulation and early signal transduction events in cytotoxic T lymphocytes inactivated by sensitive target cells. J. Immunol. **147**:3237–3243.
11. **Kuo, Y-C., and C-Y. Lin.** 1990. Recurrent herpes simplex virus type 1 infection precipitated by the impaired production of interleukin-2, alpha-interferon, and cell-mediated cytotoxicity.

J. Med. Virol. **31**:183–189.
12. **McChesney, M. B., and M. B. Oldstone.** 1987. Viruses perturb lymphocyte functions: selected principles characterizing virus-induced immunosuppression. Annu. Rev. Immunol. **5**:279–304.
13. **Oh, S. H., G. Trinchieri, S. Bandyopadhyay, and S. E. Starr.** 1990. Natural killer cell-mediated lysis of herpes simplex virus-infected fibroblasts: inability to detect soluble factors that contribute to lysis. Cell. Immunol. **127**:221–229.
14. **Pass, R. F., R. J. Whitley, J. D. Whelchel, A. G. Diethelm, D. W. Reynolds, and C. A. Alford.** 1979. Identification of patients with increased risk of infection with herpes simplex virus after renal transplantation. J. Infect. Dis. **140**:487–492.
15. **Phillips, R. E., S. Rowland-Jones, D. F. Nixon, F. M. Gotch, J. P. Edwards, A. O. Ogunlesi, J. G. Elvin, J. A. Rothbard, C. R. M. Bangham, C. R. Rizza, and A. J. McMichael.** 1991. Human immunodeficiency virus genetic variation that can escape cytotoxic T cell recognition. Nature (London) **354**:453–459.
16. **Pien, F. D., T. G. Smith, C. F. Anderson, M. L. Webel, and H. F. Taswell.** 1973. Herpesviruses in renal transplant patients. Transplantation **16**:489–495.
17. **Pircher, H., D. Moskophidis, U. Rohrer, K. Burki, H. Hengartner, and R. M. Zinkernagel.** 1990. Viral escape by selection of cytotoxic T cell-resistant virus variants *in vivo*. Nature (London) **346**:629–633.
18. **Posavad, C. M., and K. L. Rosenthal.** Unpublished data.
19. **Prabhu Das, M. R., D. S. Gridley, and J. D. Kettering.** 1991. Suppression of immune responses by herpes virus type 2-transformed murine tumor cells. Immunol. Lett. **30**:37–46.
20. **Quinnan, G. V., H. Masur, A. H. Rook, G. Armstrong, W. R. Frederick, J. Epstein, M. S. Manischewitz, A. M. Macher, L. Jackson, J. Ames, H. A. Smith, M. Parker, G. R. Pearson, J. Panillo, C. Mitchell, and S. E. Strauss.** 1984. Herpesvirus infections in the acquired immunodeficiency syndrome. JAMA **252**:72–77.
21. **Rand, K. H., L. E. Rasmussen, R. B. Pollard, A. Arvin, and T. C. Merigan.** 1976. Cellular immunity and herpesvirus infection in cardiac-transplant patients. N. Engl. J. Med. **296**:1372–1377.
22. **Rinaldo, C. R.** 1990. Immune suppression by herpesviruses. Annu. Rev. Med. **41**:331–338.
23. **Sadat-Sowti, B., P. Debre, T. Idziorek, J. M. Guillon, F. Hadida, E. Okzenhendler, C. Katlama, C. Mayaud, and B. Autran.** 1991. A lectin-binding soluble factor released by $CD8^+CD57^+$ lymphocytes from AIDS patients inhibits T cell cytotoxicity. Eur. J. Immunol. **21**:737–741.
24. **Schmid, D. S.** 1988. The human MHC-restricted cellular response to herpes simplex virus type 1 is mediated by $CD4^+$, $CD8^-$ T cells and is restricted to the DR region of the MHC complex. J. Immunol. **140**:3610–3616.
25. **Sethi, K. K., I. Stroehmann, and H. Brandis.** 1980. Human T-cell cultures from virus-sensitized donors can mediate virus-specific and HLA-restricted cell lysis. Nature (London) **286**:718–720.
26. **Siegal, F. P., C. Lopez, G. S. Hammer, A. E. Brown, S. J. Kornfeld, J. Gold, J. Hassett, S. Z. Hirshman, C. Cunningham-Rundles, B. R. Adelsberg, D. M. Parham, M. Siegal, S. Cunningham-Rundles, and D. Armstrong.** 1981. Severe acquired immunodeficiency in male homosexuals manifested by chronic herpes simplex lesions. N. Engl. J. Med. **305**:1439–1444.
27. **Torpey, D. J., M. D. Lindsley, and C. R. Rinaldo.** 1989. HLA-restricted lysis of herpes simplex virus-infected monocytes and macrophages by $CD4^+$ and $CD8^+$ T lymphocytes. J. Immunol. **142**:1325–1332.
28. **Townsend, A., and H. Bodmer.** 1989. Antigen recognition by class I-restricted T lymphocytes. Annu. Rev. Immunol. **7**:601–624.
29. **Wold, W. S. M., and L. R. Gooding.** 1991. Region E3 of adenovirus: a cassette of genes involved in host immunosurveillance and virus-cell interactions. Virology **184**:1–8.
30. **Yasukawa, M., A. Inatsuki, and Y. Kobayashi.** 1989. Differential in vitro activation of $CD4^+CD8^-$ and $CD8^+CD4^-$ herpes simplex virus-specific human cytotoxic T cells. J. Immunol. **143**:2051–2057.
31. **Yasukawa, M., T. Shiroguchi, and Y. Kobayashi.** 1983. HLA-restricted T lymphocyte-mediated cytotoxicity against herpes simplex virus-infected cells in humans. Infect. Immun. **40**:190–197.
32. **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.
33. **Yasukawa, M., and J. M. Zarling.** 1985. Human cytotoxic T cell clones directed against herpes simplex virus-infected cells. III. Analysis of viral glycoproteins recognized by CTL clones by using recombinant herpes simplex viruses. J. Immunol. **134**:2679–2682.
34. **Zarling, J. M., P. A. Moran, R. L. Burke, C. Pachl, P. W. Berman, and L. A. Lasky.** 1986. Human cytotoxic T cell clones directed against herpes simplex virus-infected cells. IV. Recognition and activation by cloned glycoproteins gB and gD. J. Immunol. **136**:4669–4673.
35. **Zarling, J. M., P. A. Moran, L. A. Lasky, and B. Moss.** 1986. Herpes simplex virus (HSV)-specific human T-cell clones recognize HSV glycoprotein D expressed by a recombinant vaccinia virus. J. Virol. **59**:506–509.