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Herpes Simplex Inhibits the Capacity of Lymphoblastoid B Cell Lines to Stimulate CD4+ T Cells

Serge Barcy and Lawrence Corey

HSV establish a lifelong persistent infection in their host even among immunocompetent human persons. The viruses use a variety of immune evasion strategies, presumably to assist persistent replication in the human host. We have observed that infection of human B lymphoblastoid cells (B-LCL) by HSV resulted in a strong inhibition of their ability to induce CD4+ T cell clone proliferation and cytokine secretion. This inhibitory effect occurs in a variety of both HSV- and HIV-specific clones from three different patients. The inhibition is observed when the Ag is provided either as a soluble protein or as a synthetic peptide and is not associated with detectable down-regulation of the MHC class II molecules or costimulatory molecules. Expression of the HSV-1 unique sequence 1 gene (US1) is necessary and sufficient to induce this inhibition of APC function. US1 gene expression also made B-LCL less susceptible to CD4+ T cell-mediated lysis. These data indicate a novel immune evasion strategy by HSV-1 in which Ag-processing cells that become infected by HSV-1 are inhibited in their ability to induce subsequent CD4+ T cell activation. The Journal of Immunology, 2001, 166: 6242–6249.

Herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) are common human pathogens that first infect and replicate in epithelial cells of mucosal membranes and then spread via sensory neurons into ganglia, where they establish a chronic latent infection (1). Reactivation of HSV from neurons and reinfection of the mucosal tissues occurs intermittently, causing recurrent cutaneous lesions despite the presence of relatively high titers of neutralizing Ab (2) and detectable HSV-specific T cell responses (3). Antigenic variation is not utilized by HSV as a mechanism of persistence. Several mechanisms have been identified by which HSV-1 inhibits recognition by CD8+ T cells. The HSV-1 infected cell protein (ICP)4 blocks the peptide transporter (TAP), preventing access of antigenic peptides into the class I pathway (4). HSV-1 has also been shown to prevent the transport of MHC class I molecules to the cell surface (5) and can block CTL-induced Ag-dependent apoptosis (6). However, a wide variety of evidence also points to the critical need for CD4+ T cells in maintaining effective and persistent cytotoxic T lymphocyte responses (7). CD4+ T cells are the initial infiltrating cells in herpetic lesions and secrete a wide variety of cytokines (8). Release of cytokines by CD4+ cells plays a major role in activation of CD8+ T cells, activation of B lymphocytes and subsequent production of specific antiviral Ab, and activation of nonspecific inflammatory cells such as NK and macrophages. Moreover, CD4+ lymphocytes constitute the predominant source of IFN-γ from blood mononuclear cells of patients with recent recurrent herpetic lesions (9). In vitro, IFN-γ partially reverses the down-regulation of MHC class I after HSV infection of epidermal cells and induces MHC class II expression (10). As such, we sought to evaluate whether HSV has developed an immune escape mechanism that could block recognition of HSV-infected cells by CD4+ T cells. The HSV-1 ICP22 product of the unique sequence 1 (US1) gene, is a nucleotidylation and phosphorylated protein with properties of a transcriptional factor required for the expression of a subset of late viral genes (11). Our current findings demonstrate that HSV-1 US1 gene expression in lymphoblastoid APC results in a dramatically reduced ability to stimulate CD4+ T cell clone proliferation and cytokine secretion.

Materials and Methods

Viruses and cells

HSV-1 (strain F), HSV-1 US1 deletion mutant (R325), HSV-1 unique sequence 3 (US3) deletion mutant (R7041), and respective repaired viruses (R4968, R7036) (12), HSV-1 unique sequence 12 (US12) deletion mutant (HV1.102), and HSV-2 (strain 186) were all grown and titered in Vero cells. HSV-2 ICP4 deletion mutant (hr259) (13) was propagated and titered on E5 cells.

All cell lines were maintained in complete medium, consisting of RPMI 1640 supplemented with antibiotics and 10% heat-inactivated bovine serum (Gemini Bio-Products, Woodland, CA). The study protocol was approved by the University of Washington Institutional Review Board.

EBV-transformed lymphoblastoid cell lines (B-LCL) and HSV-specific T cell clones EB47, EB13, ES21, and ES37 were derived as previously described (14). The T cell clones were established in culture from a skin lesion biopsy (clones EB47 and EB13) or a blood sample (clone ES21 and ES37) obtained from immunocompetent patients with genital HSV-2 infection. The clonality of each T cell line was assessed by PCR for TCR Vβ gene expression (Clontech Laboratories, Palo Alto, CA, ). HIV-specific T cell clone 9G8 has been previously described (15).

Plasmid expression vectors

The full coding sequence of US1 was amplified by PCR from HSV-1 viral DNA (strain F) by using primers (5'-GGTTGTACCCGCCGATGATG GCCGACATTTC-3') and (5'-GCCCGATCCCGCCTCCGCCG CATTTTA-3'). After a 5-min incubation at 94°C, the PCR was cycled 35 times at 94°C for 30 s, 60°C for 30 s, and 68°C for 3 min followed by a
final extension at 68°C for 7 min using the High Fidelity PCR System (Boehringer Mannheim, Indianapolis, IN). The PCR product was cloned in the pCR II TOPO vector and sequenced using dye deoxy-terminator (Applied Biosystems Prism; Applied Biosystems, Foster City, CA). The USI gene was then transferred in two fragments, Xhol-HindIII of 0.9 kb and HindIII-BamHI of 0.5 kb, into pNS vector (16) opened by Xhol-BamHI.

**Transfection**

B-LCL were transfected using LipofectAMINE (Life Technologies, Rockville, MD) according to the manufacturer’s protocol. Briefly, cells (3 \( \times 10^6 \)) were washed in serum-free RPMI 1640 and resuspended in 0.8 ml of Optitemp (Life Technologies). Ten micrograms of purified plasmid DNA was mixed with 20 \( \mu l \) of LipofectAMINE in 0.2 ml of Optitemp, incubated for 45 min at room temperature, and added to the B-LCL suspension in one well of a six-well plate. The cells were then incubated in a 5% CO\(_2\) humidified atmosphere for 5 h. After 4 h, the cells were washed and resuspended in fresh complete RPMI 1640 medium. G418 sulfate (Calbiochem, La Jolla, CA) was added on day 3 at 1 U/ml.

**Ag-specific proliferation**

B-LCL were infected for 18 h with the relevant virus at a multiplicity of infection (MOI) of 10, then washed and counted. After irradiation (10,000 rad), stimulator cells (10\(^5\) cells/ml) were mixed with T cell clones (5 \( \times 10^5 \) cells/ml) in the presence of 1 \( \mu g/ml \) of purified HSV glycoprotein B (gB) (gD) 10 min.

For peptide stimulation, irradiated cells were incubated with 10 \( \mu g/ml \) of aa 571–590 from HIV-1 envelope peptide epitope (VVGHKQLQAVR LAVERYLKD) for 1 h on ice before being washed, irradiated, and used as stimulatory cells at various dilutions.

After 3 days of culture, cells were pulsed for 16 h with 0.5 \( \mu Ci \) of \([\text{I}^3\text{H}]\)thymidine (NEN, Boston, MA). \([\text{I}^3\text{H}]\)Thymidine incorporation was determined with a liquid scintillation counter. Proliferation of T cell clones was performed in triplicate wells.

**Cytokine titration**

After 24-h culture, supernatants from triplicate wells were pooled and tested for the presence of cytokines. Measurements of human IL-2 and IFN-\(\gamma\) were analyzed using a sandwich ELISA. Samples were tested in duplicate. The coefficient of variation was always <10%. Matched pair Abs were purchased from R&D Systems (IL-2; Minneapolis, MN) and Endogen (IFN-\(\gamma\); Woburn, MA).

The lowest detection limit for the IL-2 assay is 5 and 0.5 pM/ml for the IFN-\(\gamma\) assay.

**Cytotoxicity assay**

Standard chromium release assays were performed. Briefly, autologous target B-LCL were infected for 18 h with relevant virus at an MOI of 10 in the presence of 100 \( \mu Ci \) of \( ^{51}\text{Cr} \) (NEN), washed, and counted. CD4\(^+\) T cell clones and \( ^{51}\text{Cr} \)-labeled target B-LCL were cultured together using various E:T ratios. After 4 h at 37°C, 30 \( \mu l \) of supernatant was removed and counted in Lumaplates with a TopCount scintillation counter (Packard, Meriden, CT).

For redirected cytotoxicity assay, P815 FcR-positive mouse masto-
toma cells were infected at an MOI of 10 for 18 h in the presence of \( ^{51}\text{Cr} \). The labeled target cells were then mixed with T cells at various E:T ratios. Purified OKT3 Ab was added at a final concentration of 1 \( \mu g/ml \).

Percent specific lysis was calculated as: 100 \( \times \) (cpm released with effectors – cpm released alone)/[cpm released by detergent – cpm released alone]. Spontaneous release was always <20% of maximum release.

**Flow cytometry**

Standard procedures were used for flow cytometric analysis. B-LCL (1 \( \times 10^6 \)) were resuspended in PBS containing 1% FBS and 0.1% NaN\(_3\). The cells were incubated at 4°C for 30 min with either primary or PE-labeled mAbs of the appropriate specificity. When relevant, cells were washed and incubated for an additional 30 min at 4°C with PE-labeled goat anti-mouse Ig (Sigma, St. Louis, MO).

MHC class II molecule expression was evaluated using mAb B7.21 specific for the HLA-DP framework determinants, mAb SPV-L3 specific for the HLA-DQ framework, and mAb L-243 specific for the HLA-DR framework (14). Abs specific for CD80 and CD86 molecules were purchased from Becton Dickinson (Mountain View, CA).

Cytoplastic staining was performed as previously described (17). Cells were fixed with 3.7% paraformaldehyde and permeabilized using a 0.1% Nonidet P-40 solution in PBS. The cells were then incubated with the rabbit antiserum R77 (12) and stained with an FITC-labeled goat anti-rabbit antiserum (Vector Laboratories, Burlingame, CA).

Relative fluorescence intensities were measured with a FACScalibur (Becton Dickinson). Data were analyzed using WinMDI software (Joseph Trotter, The Scripps Clinic, La Jolla, CA).

**Results**

**HSV-1 infection of B-LCL inhibits Ag-specific T cell activation**

Human B cells immortalized by EBV (B-LCL) have been widely used as APC. We sought to evaluate whether B-LCL infected by HSV-1 were impaired in their ability to efficiently stimulate CD4\(^+\) T cell clones. Initially, we used HSV-specific CD4\(^+\) T cell clones that were isolated from two different donors with genital herpes. Two clones, EB47 and EB13, were derived from the expansion of lesion-infiltrating T cells from a skin biopsy of an HSV-2 buttock lesion. The clones were CD3\(^+\)/CD4\(^+\)/CD8\(^-\) and were determined to recognize the HSV glycoproteins gB and gD, respectively. Two other clones, ES21 and ES37, were isolated from a peripheral blood sample from a different patient with genital HSV-2 infection and were found to be specific for the viral encoded gD. Following stimulation with their respective Ag, the cytokine secretion level for each T cell clone varied from low (clone ES21) to high (clone EB13). The pattern was consistent between experiments (Table I).

| Table I. HSV-1 infection of B-LCL inhibits their capacity to induce CD4\(^+\) T cell activation |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|
|                                | Uninfected | Uninfected + Ag | HSV-1 | HSV-1 + Ag |
| **Clone ES21**                  |            |                 |      |             |
| Proliferation (mean cpm ± SD)   | 713 ± 399  | 37,464 ± 2,212 | 128 ± 19 | 155 ± 44 |
| (pg/ml)                         | <LLD       | <LLD            | <LLD  | <LLD         |
| INF-\(\gamma\) (pg/ml)          | 369        | 19,179 ± 2,062 | 2,073 ± 13 | 2,590 ± 228 |
| **Clone EB47**                  |            |                 |      |             |
| Proliferation (mean cpm ± SD)   | 504 ± 21   | 1,024 ± 153    | 218 ± 24 | 430 ± 95   |
| (pg/ml)                         | <LLD       | 5,757 ± 337    | 72    | 156         |
| INF-\(\gamma\) (pg/ml)          | 159        | 2,101 ± 17     | 4     | 135         |
| **Clone EB13**                  |            |                 |      |             |
| Proliferation (mean cpm ± SD)   | 1,024 ± 153| 342             | 72    | 156         |
| (pg/ml)                         | <LLD       | 3,000           | 157   | 652         |

\( ^a \) B-LCL infected overnight were used as stimulator cells to activate HSV-specific CD4\(^+\) T cells in the presence of purified gB (clone EB47) or gD (clones EB13 and ES21) at a final concentration of 1 \( \mu g/ml \). The data are representative of three independent experiments performed in triplicate. Cytokine secretion was measured at 24 h in pooled supernatants from triplicate cultures. The coefficient of variation for cytokine titration was >10%.

\( ^b \) Clone ES21 does not secrete any detectable IL-10 or IL-4.

\( ^c \) LLDD, Below the lower limit of detection.
To assess the effect of HSV infection on Ag presentation function, B-LCL were infected at an MOI of 10, cultured overnight, and then tested in a proliferation assay. As shown in Table I, HSV-1-infected autologous B-LCL were much less potent in stimulating Ag-specific T cell proliferation than uninfected B-LCL were. Cell proliferation measured by [3H]thymidine incorporation on day 3 is a distal measure of APC-T cell interactions that have occurred much earlier. Therefore, we also evaluated whether IL-2 and IFN-γ secretion in culture supernatant was concordant with the proliferative activity under identical culture conditions. As expected, uninfected B-LCL have the stimulatory capacity to induce a strong cytokine release in the culture supernatant. In contrast, supernatants obtained using HSV-infected B-LCL as stimulator cells contained much lower cytokine titers, suggesting that HSV-infection of B-LCL greatly affects their capacity to stimulate CD4+ T cell clones both at the level of proliferation and for IL-2 or IFN-γ secretion.

**HSV immediate-early gene expression is required to induce inhibition of CD4+ T cell activation**

We next sought to define viral gene function associated with this inhibitory effect. UV-inactivated HSV-2 did not induce inhibition of CD4+ T cell proliferation as compared with infectious HSV-2 (Table II). As such, we directed our efforts at evaluating viral-specific genes that were not contained in the viral particle. We used an HSV-2 ICP4 mutant (hr259). Since ICP4 is strictly required for expression of both early and late proteins, this mutant expresses only immediate-early proteins (ICP0, ICP6, ICP22, ICP27, and ICP47) (13). As clone EB47 is directed against the viral encoded gB, a late gene, we prevented any potential competition between soluble Ag and viral particles by preincubating the B-LCL with the glycoprotein before infection with the ICP4 mutant. As shown in Table II, B-LCL infection with the ICP4 mutant hr259 resulted in a strong inhibition of both the T cell proliferation and cytokine secretion. Although some IFN-γ was detected with HSV-2-infected B-LCL, the amount was significantly less than that induced by uninfected B-LCL. These data suggested that expression of an immediate-early HSV protein other than ICP4 was associated with this inhibition of the T cell activation.

**The HSV-1 immediate-early gene US1 is required to induce inhibition of CD4+ T cell activation**

We then tested whether the immediate-early protein ICP47, encoded by the HSV-1 US12 gene, could affect B-LCL APC function. As shown in Table III, B-LCL infected by the HSV-1 US12 deletion mutant (HV1.102) still lost their capacity to fully activate the CD4+ T cell clone EB47 in response to the purified gB. This inhibition was observed at the level of T cell proliferation as well as with cytokine secretion, suggesting that US12 gene expression is not required to induce the inhibition of the T cell activation.

We next evaluated whether the immediate-early protein ICP22, encoded by the US1 gene, has any inhibitory effect on the B-LCL APC function. For these experiments, we used a HSV-1 US1 deletion mutant (R325). Another HSV-1 deletion mutant (R7041/US3−) and respective repaired viruses (R4968/US1+ and R7036/US3−) were used as controls in the assays. As shown in Fig. 1, following infection with the US1 deletion mutant, autologous B-LCL were able to induce a strong T cell proliferation (A) and IL-2 or IFN-γ secretion (B and C) in response to the gB (clone EB47) or gD (clone ES37). This was observed when the Ag was provided either before or after the cells were infected. Moreover, even without addition of any soluble Ag, a significant T cell activation was induced in the presence of B-LCL infected with US1 mutant, suggesting that under these conditions the endogenous viral glycoproteins gB and gD were efficiently processed and presented to the respective CD4+ T cell clones. In contrast, infection with the US3 deletion mutant or repaired viruses resulted in a poor T cell activation similar to that seen previously with wild-type HSV. These experiments indicated that the impaired stimulatory capacity of HSV-infected B-LCL appeared to be predominantly due to HSV-1 US1 gene expression.

**The HSV-1 viral protein ICP22 is responsible for the lack of T cell activation**

We next investigated whether the US1 gene product ICP22 was associated with the observed inhibition of the B-LCL APC function. The US1 gene was amplified by PCR from a DNA extract obtained from wild-type HSV-1 (strain F), and the PCR product was cloned into the eukaryotic cell expression vector pNS. Following amplification and purification, the expression vector was transfected into B-LCL using LipofectAMINE.

ICP22 protein expression was assessed using an intracellular staining technique. Uninfected, HSV-1-infected, and US1-transfected B-LCL were stained using a ICP22-specific rabbit antiserum and analyzed by flow cytometry. As shown in Fig. 2, following the same culture conditions as previously described for the T cell proliferation assay, ICP22 was detected in HSV-1-infected B-LCL. ICP22 was also detected with similar fluorescence intensity in all B-LCL after stable transfection and G418 selection. To

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**Table II. HSV-2 ICP4 deletion mutant (hr259) inhibits B-LCL ability to stimulate the gB-specific CD4+ T cell clone EB47**

<table>
<thead>
<tr>
<th></th>
<th>Cytokine Secretion (pg/ml)</th>
<th>Proliferation* (mean cpm ± SD)</th>
<th>IL-2</th>
<th>IFN-γ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uninfected</td>
<td>&lt;LLDb</td>
<td>315 ± 105</td>
<td>&lt;LLDb</td>
<td>8</td>
</tr>
<tr>
<td>gB + Uninfected</td>
<td>13,145 ± 2,674</td>
<td>248 ± 23</td>
<td>994</td>
<td>4,060</td>
</tr>
<tr>
<td>UV-inactivated HSV-2</td>
<td>14,070 ± 1,032</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>gB + UV inactivated HSV-2</td>
<td>2,366 ± 222</td>
<td>101</td>
<td>905</td>
<td></td>
</tr>
<tr>
<td>Infectious HSV-2</td>
<td>345 ± 48</td>
<td>49</td>
<td>279</td>
<td></td>
</tr>
<tr>
<td>gB + infectious HSV-2</td>
<td>2,722 ± 196</td>
<td>101</td>
<td>905</td>
<td></td>
</tr>
<tr>
<td>hr259</td>
<td>773 ± 196</td>
<td>101</td>
<td>135</td>
<td></td>
</tr>
<tr>
<td>gB + hr259</td>
<td>398 ± 44</td>
<td>95</td>
<td>170</td>
<td></td>
</tr>
</tbody>
</table>

*a B-LCL were incubated in the presence of the purified HSV gB (10 μg/ml) prior to infection. After being cultured overnight, the infected B-LCL were used to activate T cells from clone EB47. Results shown are representative of three independent experiments. Cytokine secretion was measured at 24 h in pooled supernatants from triplicate cultures. The coefficient of variation for cytokine titration was >10%.

*b Below the lower limit of detection.

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**Table III. HSV-1 US12 mutant (HV1.102) inhibits B-LCL capacity to stimulate CD4+ T cell clone EB47**

<table>
<thead>
<tr>
<th></th>
<th>Cytokine Secretion (pg/ml)</th>
<th>Proliferation* (mean cpm ± SD)</th>
<th>IL-2</th>
<th>IFN-γ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uninfected</td>
<td>&lt;LLDb</td>
<td>1643 ± 314</td>
<td>&lt;LLDb</td>
<td>3</td>
</tr>
<tr>
<td>Uninfected + gB</td>
<td>621 ± 282</td>
<td>137 ± 23</td>
<td>18</td>
<td>135</td>
</tr>
<tr>
<td>HSV-1</td>
<td>178 ± 39</td>
<td>114 ± 9</td>
<td>43</td>
<td>102</td>
</tr>
<tr>
<td>HSV-1 + gB</td>
<td>276 ± 68</td>
<td>114 ± 9</td>
<td>43</td>
<td>102</td>
</tr>
</tbody>
</table>

*a B-LCL infected overnight were used as stimulator cells to activate gB-specific CD4+ T cells from clone EB47 in the presence of purified gB (1 μg/ml). Results shown are representative of three independent experiments. Cytokine secretion was measured at 24 h in pooled supernatants from triplicate cultures. The coefficient of variation for cytokine titration was <10%.

b LLD, Below the lower limit of detection.
assess whether ICP22 protein expression alone results in the observed inhibition, drug-resistant B-LCL expressing the ICP22 viral protein were used as APC in a T cell proliferation assay. As shown in Fig. 3, B-LCL transfected with the control vector coding for the α isoform of the mouse CD8 protein were able to efficiently process and present soluble Ag to different CD4⁺ T cell clones. In contrast, a strong inhibition of T cell clone proliferation (Fig. 3A) as well as cytokine secretion (Fig. 3, B and C) was observed in the presence of the HSV-1 US1-transfected B-LCL. US1-transfected B-LCL fail to efficiently present synthetic peptide to an HIV-specific CD4⁺ T cell clone

We further investigated whether ICP22 expression was affecting the B-LCL Ag presentation function at the level of Ag processing or presentation. We used a previously described HIV-specific CD4⁺ T cell clone (9G8), which recognizes a known peptide epitope in gp160 protein (15). Corresponding autologous B-LCL were transfected with either the HSV-1 US1 gene or the CD8 α control vector. The cells were kept under G418 selection for 10 days to ensure that only transfected B-LCL were present in the remaining culture. The drug-resistant B-LCL were preincubated with the synthetic HIV-1 envelope peptide and then washed to prevent T-T cell activation during the proliferation assay. As seen in Fig. 4, US1-transfected B-LCL are again much less potent than vector control-transfected B-LCL in stimulating T cell proliferation (A) and cytokine secretion (B and C). Although some IFN-γ secretion could be detected, the level of secretion dropped quickly following the APC dilutions.

**US1 gene transfection does not induce modulation of either MHC class II or costimulatory molecules CD80 and CD86 expression on B-LCL.**

We also assessed whether HSV-1 US1 transfection in B-LCL induced modulation of cell surface proteins. Because transfected B-LCL were less potent in presenting synthetic peptide to a CD4⁺ T cell clone, we first looked at the expression of different MHC class II molecules (DR, DQ, and DP). As shown in Fig. 5, analysis by flow cytometry revealed no detectable difference between US1-transfected cells and the vector control transfectant. In humans, a decreased T cell response to recall Ags is often associated with depressed costimulatory receptor expression (18, 19). Therefore, we analyzed whether US1 transfection could affect the level of expression of the costimulatory molecules CD80 and CD86. Again, we could not detect any significant modulation in the cell surface expression level for either molecule.
We next evaluated whether US1 gene expression could also affect CD4+ T cell-mediated lytic activity. For these experiments, we used the HIV-specific T cell clone 9G8 because of its high and stable lytic activity. B-LCL were infected with the US1 deletion virus or the corresponding repaired virus at a MOI of 10 and cultured overnight. The next day, the cells were incubated with the relevant synthetic peptide and washed before being used in a chromium release assay. As shown in Fig. 6A, B-LCL infected with the deletion mutant exhibited higher lytic activity than when infected with the repaired virus. In agreement with this observation, B-LCL transfected with HSV-1 US1 gene (Fig. 6B) also resulted in a similar decrease of the T cell lytic activity.

Because it seemed that US1 gene expression could be correlated with a decrease of the observed lytic activity, we sought to evaluate whether the ICP22 expression resulted in a lower T cell killing performance or a higher target cell resistance to lysis. To assess that question, we used a redirected lysis assay. In this assay, the cytotoxic activity is triggered using an anti-OKT3 Ab independently of any Ag recognition or accessory signal influence. P815 cells were infected following the same conditions as previously described for the B-LCL. As shown in Fig. 6C, the percent lysis observed for the US1 mutant-infected cells was again higher than with the repaired virus.

Taken together, these results suggest that expression of the HSV-1 US1 gene in B-LCL or P815 cells lowers their susceptibility to CD4+ T cell-mediated lysis.

Discussion

One of the most central issues in the immunobiology of human herpesviruses is their ability to evade the immune response. Several studies have demonstrated the important role that both HSV-specific CD4+ and CD8+ T cells play in the clearance of virus from lesions and in the healing of these lesions. Several different
mechanisms have been described by which the virus can interfere with the CD8\(^{+}\)-mediated T cell response. It is well documented that viral glycoprotein-specific CD4\(^{+}\) T cell clones proliferate and secrete lymphokines when stimulated with UV light-inactivated HSV in the presence of autologous PBL (20, 21, 22). To our knowledge, no virally encoded function has been previously identified that interferes with recognition of HSV-infected cells by human CD4\(^{+}\) T cells.

In this paper, we characterized a new immune evasion mechanism used by HSV to interfere with the CD4\(^{+}\)-mediated cellular immune response. We have demonstrated that B-LCL infected by live HSV are strongly inhibited in their ability to induce CD4\(^{+}\) T cell clone proliferation and IL-2 or IFN-\(\gamma\) secretion. This inhibitory effect does not seem to be restricted to a particular type of CD4\(^{+}\) T cell clone or B-LCL line, as this result was reported for a total of five different clones from three different patients. Moreover, we have shown that T cell susceptibility to this inhibition was not restricted to HSV-specific T cell clones in that an HIV-specific clone also shows similar inhibition of its activation.

We reasoned that this effect on Ag presentation appeared to be related to early gene expression by the virus and demonstrated for HSV-1 that the immediate-early US1 gene was necessary and sufficient to induce the APC function inhibition. The HSV-1 ICP22, the product of the US1 gene, is a nucleotidylated and phosphorylated protein with properties of a transcriptional factor required for the expression of a subset of late viral genes (11). The coding domain of the US1 gene encodes two proteins, a full-length protein, ICP22, and a protein called US1.5 which is initiated from methionine 147 of ICP22 and which is colinear with the remaining portion of that protein. The two corresponding mRNAs are expressed by their own independent promoter (23). Our data do not allow us to determine which of either the ICP22 or US1.5 proteins is responsible for the APC function inhibition. Experiments to clarify that point are currently in progress.

Previous studies have reported that HSV-1 infection of monocytes and more recently of dendritic cells resulted in an impairment of their capacity to stimulate T cells. Following HSV-1 infection, human monocytes have been shown to lose their ability to induce resting T cell proliferation in response to superantigen (24). Similarly, HSV-1-infected human dendritic cells were unable to produce cytokines and were poorly stimulatory for allogeneic T cells (25). In this latter case, the virus was found to inhibit dendritic cell maturation. In our experiments, we have demonstrated that following the expression of the viral protein ICP22, HSV-1 is able to strongly inhibit the capacity of B-LCL to trigger an Ag-specific T cell activation. The inhibition is observed when the Ag is provided either as a soluble protein or as a synthetic peptide.

The function of MHC class II molecules is to sample exogenous Ags for presentation to CD4\(^{+}\) T cells. Newly synthesized MHC class II heterodimers associate with the invariant chain (Li) in the endoplasmic reticulum (26). Once the complex reaches the endosomal system, Li is degraded leaving the class II binding site available for binding antigenic peptide (27). The peptide loading process is facilitated by the presence of H2-DM molecules involved in the dissociation of Li fragments from the class II peptide binding groove (28). Previous studies have demonstrated that the principle means of presenting a soluble peptide on human B cells involves peptide loading directly onto surface class II molecules (29, 30). Therefore, exogenously supplied peptides bypass the intracellular Ag-processing pathway. As such, inhibition of the B-LCL ability to stimulate CD4\(^{+}\) T cell clones appears to be the result of an Ag presentation rather than an Ag processing deficiency. Although we have yet to define the exact mechanisms of APC inhibition by HSV, ICP22 protein expression did not affect cell surface expression of any of the MHC class II molecules (DR, DQ, and DP) or costimulatory molecules like CD80 and CD86. However, the possibility cannot be excluded that other costimulatory molecules, like the recently described B7h (31), could play a role in the observed inhibition.

During T cell activation, engagement of receptors triggers an active accumulation of molecules at the interface of the T cell and the APC, which then increases the overall amplitude and duration of T cell signaling. This process is actively driven by the cytoskeleton (32). It has been shown that following HSV-1 infection, an early viral function induces cytoskeletal alterations (33). Therefore, it is tempting to speculate that US1 can prevent the triggering of a full, sustained T cell response by interfering with cytoskeletal rearrangements that allow the relay of extracellular stimulatory signals to the nucleus. The observed inhibition of T cell activation could also result from US1 interference with a signal transduction pathway. This could be achieved by decreasing the expression of essential components of a pathway, as was recently described for another herpesvirus, the human CMV (34). Experiments to define these hypotheses appear to be warranted.

HSV-specific cytotoxic activity in a bulk T cell population isolated from skin lesion biopsy has been shown to be associated with CD8\(^{+}\) as well as CD4\(^{+}\) T cells. Lesion-infiltrating CD4\(^{+}\) T lymphocytes may exert immune control over HSV reactivation not only through the secretion of cytokines but also through their lytic activity. HSV-1 is known to be able to inhibit CD4\(^{+}\)-mediated lysis of human fibroblasts through a mechanism not yet completely understood (35). We have found that following either viral infection or US1 gene transfection, B-LCL become less susceptible to lysis by CD4\(^{+}\) T cells. Moreover, in a redirected lysis assay, we observed that HSV-1-infected P815 cells also become less susceptible to T cell-induced lysis. A recent report showing that HSV-1 ICP22 protein can partially prevent Hep-2 cell-induced apoptosis (36) could provide an explanation for our observations.

It is thus of great interest that in mice, HSV-1 US1 deletion mutant failed to cause death following intracerebral, i.e., or intravaginal inoculation (37) as compared with the parental virus. Our studies suggest an immunological explanation for this observation.
A. Either HSV-1 US1 deletion mutant or the corresponding repaired virus (used in a chromium release assay against autologous B-LCL infected with mediated lysis. T cells from the HIV-1 envelope-specific clone 9G8 were CD8 t

tion of cytokine levels may decrease the ability of the immune 
mus using FcR-positive P815 cells as target (C). Cells infected with either 
HSV-1 US1 deletion mutant or repaired virus were cultured overnight 
before being mixed with T cells at various E:T ratios. Killing activity was also measured in a redirected lysis assay using FeR-positive P815 cells as target (C). Cells infected with either 
HSV-1 US1 deletion mutant or repaired virus were cultured overnight 
before being mixed with T cells at various E:T ratios in the presence of 
purified OKT3 Ab (1 µg/ml). This pattern of results was obtained in three 
other experiments. Error bars represent the SD of triplicate results.

The enhanced APC function associated with the deletion mutant 
would result in an enhanced host response and hence lower 
mortality. In summary, we have shown that upon HSV infection, B-LCL 
are much less potent in inducing IFN-γ secretion in CD4+ T cells. In addition, the IL-2 secretion was also found to be strongly 
hindered. We propose that, upon HSV infection, the overall reduction 
of cytokine levels may decrease the ability of the immune 
response to control viral spread at the onset of an outbreak because of 
the subsequent deficiency in antiviral cytokines, lower help for 
CD8+ T cells, or decreased local neutralizing Abs.

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FIGURE 6. ICP22 expression protects target cells from CD4+ T cell-
mediated lysis. T cells from the HIV-1 envelope-specific clone 9G8 were 
used in a chromium release assay against autologous B-LCL infected with 
either HSV-1 US1 deletion mutant or the corresponding repaired virus (A) 
or against B-LCL transfected with either pNS HSV-1 US1 or the pNS CD8 
control vector (B). Target cells were incubated with the HIV-1 envelope 
synthetic peptide before being washed and mixed with T cells at various 
E:T ratios. Killing activity was also measured in a redirected lysis assay 
using FeR-positive P815 cells as target (C). Cells infected with either 
HSV-1 US1 deletion mutant or repaired virus were cultured overnight 
before being mixed with T cells at various E:T ratios in the presence of 
purified OKT3 Ab (1 µg/ml). This pattern of results was obtained in three 
other experiments. Error bars represent the SD of triplicate results.


