JOURNAL OF VIROLOGY, Dec. 1996, p. 8747–8751 Vol. 70, No. 12
0022-538X/96/$04.00+0
Copyright © 1996, American Society for Microbiology

Herpes Simplex Virus Type 2 Inhibition of Fas Ligand Expression

SCOTT SIEG,[1] ZAFER YILDIRIM,[1] DAWN SMITH,[1] NOBUHIKO KAYAGAKI,[2] HIDEO YAGITA,[2] YUNG HUANG,[1] AND DAVID KAPLAN[1]*

Department of Pathology, Case Western Reserve University, Cleveland, Ohio 44106-4943,[1] and Department of Immunology, Juntendo University School of Medicine, Tokyo 113, Japan[2]

Received 18 June 1996/Accepted 5 September 1996

Herpes simplex virus types 1 and 2 (HSV-1 and HSV-2) are common human pathogens. In this report we demonstrate the capacity of HSV-2, but not HSV-1, to inhibit the activity and cell surface expression of Fas ligand, an important molecule involved in T-cell apoptosis and cell-mediated cytotoxicity. Cells infected with HSV-2 retained Fas ligand intracellularly instead of expressing it on the cell surface. Addition of anti-Fas antibodies markedly inhibited HSV-2 viral production, suggesting that the capacity of the virus to regulate Fas ligand expression, and thereby programmed cell death, may represent a powerful mechanism for the virus to enhance viral replication.

Because cytotoxicity is an important modality in viral immunity, the cytotoxic process has been targeted for disruption by many viruses. For instance, adenoviruses prevent surface expression of major histocompatibility complex (MHC) class I molecules on infected cells, thereby limiting antigenic recognition by cytotoxic T cells (1, 2, 5, 17, 36). Similarly, hepatitis B virus and human immunodeficiency virus inhibit cytotoxicity by producing variant peptides of immunogenic epitopes; the variant peptides bind to MHC molecules but antagonize the initiation of T-cell-mediated cytotoxicity (3, 21).

Herpes simplex viruses type 1 and type 2 (HSV-1 and HSV-2) are important human pathogens that cause persistent and recurrent infections (7, 22, 39). As with adenoviruses, HSV-1 and HSV-2 have been shown to prevent the expression of MHC class I molecules on the cell surface and thereby inhibit antigenic recognition by cytotoxic T lymphocytes (15, 42). The mechanism mediating this effect involves the binding of ICP47, an intermediate-early protein of HSVs, to the transporter associated with antigen processing, known as TAP (12, 16).

Besides the capacity of viruses to disrupt cytotoxicity by preventing antigenic recognition, viruses also have the capacity to infect T lymphocytes and directly affect the machinery of cytotoxicity (6, 9, 25, 26). For instance, parainfluenza virus type 3 inhibits granzyme B mRNA in infected T lymphocytes (35). Granzyme B is a component of cytotoxic granules important in mediating cytotoxicity by granular exocytosis; consequently, parainfluenza virus type 3 infection of T cells inhibits their capacity to mediate cytotoxicity (35).

In addition to the exocytosis of lytic granules, cytotoxicity of Fas-bearing cells is caused by the ligation of Fas with Fas ligand on effector cells (14, 18, 23). These two pathways have been shown to account for essentially all cytotoxicity mediated by T lymphocytes.

The Fas-Fas ligand pathway is also important as a regulator of apoptosis. Apoptosis of cells infected with viruses is a possible mechanism to decrease viral production since viruses require live cells for their own replication (35a, 38). Analogous to the evasion of cytotoxicity by viruses, several viruses (including adenoviruses, herpesviruses, and poxviruses, among others) possess mechanisms to inhibit cellular apoptosis (30).

We have assessed the possibility that HSV-1 or HSV-2 influences Fas ligand activity and have found that HSV-2 but not HSV-1 potently inhibits Fas ligand surface expression in infected cells and thereby suppresses Fas ligand-mediated cell death.

* Corresponding author. Mailing address: Department of Pathology, Case Western Reserve University, Biomedical Research Building, Room 926, 2109 Adelbert Rd., Cleveland, OH 44106-4943.

MATERIALS AND METHODS

Viruses. Respiratory synctial virus, HSV-1, and HSV-2 were obtained from patient samples, grown on CV-1 monolayers or ZY(LCL) cells, and typed by immunohistology. Human herpesviruses 6 and 7 were generously supplied by P. Pellett (Centers for Disease Control and Prevention, Atlanta, Ga.) and R. Gallo (National Institutes of Health, Bethesda, Md.). Influenza virus was obtained from J. Nedrud, and vesicular stomatitis virus was obtained from S. Vande Pol (both of Case Western Reserve University, Cleveland, Ohio). HSV-1 and HSV-2 were titered on CV-1 monolayers.

Cell lines. K562 and ZY(LCL) cells were maintained in RPMI 1640 with 10% fetal bovine serum. CV-1 monolayers were grown in Dulbecco modified Eagle medium with 10% fetal bovine serum. K562 cells infected with a retrovirus that codes for the expression of human Fas ligand (KFL) were obtained with the cDNA for human Fas ligand and the LXSN vector (35b). Jurkat and K562 cells were maintained in culture with RPMI 1640 supplemented with 10% fetal bovine serum, glutamine, and antibiotics. K562 cells were infected with a recombinant retrovirus, derived from pLXSN generously supplied by Dusty Miller, University of Washington. An expression construct for human Fas ligand was produced by recombining the coding sequence (obtained in Bluescript from Shigekazu Nagata, Osaka Bioscience Institute) with pLXSN by using the *Eco*RI-*Bam*HI site. The packaging cell lines PE501 and PA317 were used consecutively to obtain recombinant, infectious virus coding for human Fas ligand. KFL cells were obtained by culturing the cells in neomycin and subcloning.

DNA fragmentation assay. DNA fragmentation assays (24) were performed as previously described (35). Jurkat cells (10^6 ml^{-1} in RPMI 1640 with 10% fetal bovine serum) were labeled with tritiated thymidine (2.5 μCi ml^{-1}) for 4 h at 37°C. For measurement of Fas-dependent killing by peripheral blood mononuclear cell (PBMC) effectors, 2 μg of either an anti-Fas monoclonal antibody that inhibits apoptosis mediated through the Fas ligand-Fas pathway (Immunotech, Westbrook, Maine) or an isotype-matched control monoclonal antibody per ml was added to the Jurkat cells during the last hour of this incubation. The target cells were washed and added to effector cells along with 500 ng of antibody. After a 14-h incubation at 37°C, the cells were harvested and their levels of radioactivity were determined by liquid scintillation counting. Percent specific apoptotic cell death was calculated by subtracting the experimental counts per minute from the spontaneous counts per minute, dividing this number by the spontaneous counts per minute, and multiplying by 100. Fas-dependent killing was determined by subtracting the value for cytotoxicity with anti-Fas inhibitory antibodies from the value for cytotoxicity with the control antibody.

Reverse transcriptase PCR. Reverse transcriptase PCR was performed as previously described (10) with slight modifications. Total RNA was isolated from PBMC and reverse transcribed to cDNA with an oligo(dT) primer and Moloney

murine leukemia virus reverse transcriptase (Gibco-BRL, Gaithersburg, Md.) at 37°C for 60 min. Two micrograms of RNA was amplified by PCR specific for Fas ligand with the following primers: 5′ CAGCTCTTCCACCTACAGAAGG (forward direction) and 5′ AGATTCCTCAAAATTGACCAGAGAGAG (reverse direction). β-Actin primers were also used (35). Ten-millimolar concentrations of nucleotides were added to the RNA and the primers, and 2.5 U of *Taq* polymerase was added to the reaction mixture after the reaction temperature reached 94°C. The conditions for the PCR follow: 40 cycles of 94°C for 1.5 min, 60°C for 1 min, and 72°C for 2 min. The products were resolved on a 1.2% agarose gel and visualized with ethidium bromide under UV light.

Flow-cytometric analysis. Isolated human PBMC were processed for flow-cytometric analysis of surface markers as previously described (35). To assess Fas ligand surface expression, we used a biotinylated mouse monoclonal antibody specific for human Fas ligand, NOK-1 (19), and biotinylated control immunoglobulin G1 (IgG1) antibodies (Dako, Carpinteria, Calif.). Monoclonal antibodies specific for human CD40 and CD54 were obtained from Immunotech. For total cellular expression of Fas ligand, cells were fixed in 0.25% paraformaldehyde for 1 h at 4°C and then frozen at −70°C in phosphate-buffered saline (PBS) supplemented with 1% bovine serum albumin (BSA) and 10% dimethyl sulfoxide. Following freezing, the cells were thawed at 37°C, washed in PBS containing 0.1% BSA, resuspended in PBS supplemented with 0.2% Tween 20, and kept at 37°C for 15 min. The same volume of PBS containing 2% fetal bovine serum and 0.1% sodium azide was added. The cells were washed and resuspended in PBS supplemented with 0.01% HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid), 0.1% BSA, 10 μg of streptavidin per ml, 10% dialyzed human plasma, and 5% clarified mouse ascites containing mouse IgG1 and IgG2a. After overnight incubation at 4°C, the cells were washed and resuspended in PBS supplemented with 10% dialyzed human plasma and 1 μM biotin for 30 min at 4°C. The cells were incubated with biotinylated NOK-1 antibody for 45 min at 4°C, washed in PBS containing 0.5% BSA and 0.1% sodium azide, and then incubated with streptavidin red for 30 min at 4°C. The cells were washed and analyzed for bound fluorescence by flow cytometry.

RESULTS

Inhibition of Fas ligand activity by HSV-2. To investigate the potential of HSV-1 and HSV-2 to regulate Fas ligand activity, we examined the effects of viral infection on a human Fas ligand-transfected K562 cell line, KFL. We found that HSV-2, but not HSV-1, dramatically inhibited the ability of the KFL cells to mediate cytotoxicity against the Fas antigen-expressing Jurkat cell line (Fig. 1). After a single day of exposure to HSV-2, cytotoxicity was markedly inhibited. With another day of incubation, Fas ligand-mediated killing was absent. Additional HSV-1 and HSV-2 clinical isolates gave similar results (data not shown).

Although HSV-1 and HSV-2 were equally able to cause infection of KFL cells, as determined by immunohistology (with approximately 40% of the cells expressing viral proteins after 2 days in culture with either virus), only HSV-2-infected cells demonstrated a defect in Fas ligand-dependent killing. Moreover, no other virus tested (respiratory syncytial virus, influenza A virus, parainfluenza virus type 3, vaccinia virus, Epstein-Barr virus, and vesicular stomatitis virus) inhibited Fas ligand (data not shown).

Because Fas ligand is expressed in T lymphocytes and because HSV-2 is known to be able to infect T cells (4, 20, 29, 32), we tested whether the virus could inhibit Fas ligand activity in these cells. PBMC were activated with anti-CD3 monoclonal antibodies, and after 3 days of culture the cells were infected with HSV-2. After an additional 3 days, the cells were assessed for Fas ligand activity (Fig. 2). HSV-2-infected PBMC demonstrated a marked reduction in Fas-dependent killing activity. In contrast, human herpesvirus 6B, which also readily infects T cells (11, 34), was unable to inhibit Fas ligand activity (Fig. 2). We also tested human herpesviruses 6A and 7 and found that activated PBMC infected with these viruses did not demonstrate any inhibition (data not shown). Human herpesviruses 6A, 6B, and 7 infected approximately 20% of the activated PBMC, as judged by cytopathic effect.

Inhibition of Fas ligand surface expression by HSV-2. To characterize the defect in Fas ligand activity associated with HSV-2-infected KFL cells, we examined mRNA levels for Fas ligand and β-actin by reverse transcriptase PCR analysis. Data shown in Fig. 3 demonstrate that the level of Fas ligand mRNA was not affected by either HSV-1 or HSV-2 infection. Thus, the defect in Fas ligand activity associated with HSV-2 was not related to transcriptional regulation.

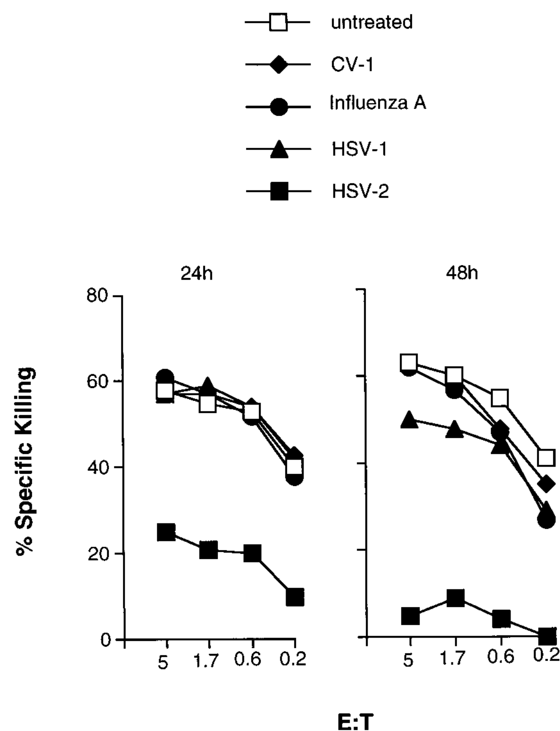

FIG. 1. Inhibition of Fas ligand activity by HSV-2. KFL cells were cultured with the indicated viruses at 3 PFU per cell for 24 and 48 h and then assayed for Fas ligand activity in a DNA fragmentation test. Untransfected K562 cells did not kill the Jurkat targets. Cytopathic effects due to viral production and subsequent infection of target cells accounted for less than 10% of the observed cytotoxicity. E:T, effector-to-target cell ratio.

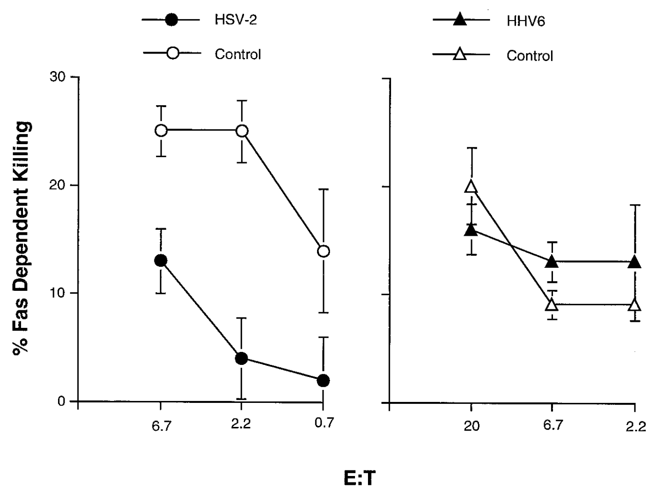

FIG. 2. Inhibition of Fas ligand activity in PBMC by HSV-2. PBMC from healthy adult volunteers were activated with anti-CD3 and infected 3 days later with either HSV-2 (left panel) or human herpesvirus 6b (HHV6) (right panel). Fas ligand activity was assessed after an additional 3 days of incubation. E:T, effector-to-target cell ratio.

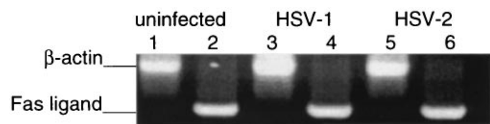

FIG. 3. Fas ligand mRNA levels in HSV-1- and HSV-2-infected KFL cells. KFL cells were infected at 3 PFU per cell and incubated for 24 h. RNA was isolated and assessed for Fas ligand and β-actin messages.

To ascertain cell surface protein expression of Fas ligand in HSV-1- and HSV-2-infected KFL cells, we analyzed the infected cells with a specific monoclonal antibody and by flow cytometry (19). Figure 4 demonstrates that the cell surface expression of Fas ligand was markedly inhibited by HSV-2 (91%) but not by HSV-1. Thus, the lack of cell surface expression of Fas ligand explains the observed functional defect. HSV-2-mediated inhibition of Fas ligand surface expression was specific; the expression of four other cell surface molecules was not inhibited: CD40 and CD54 (Fig. 4) and CD55 and CD69 (not shown). These findings indicate that the defect in Fas ligand surface expression mediated by HSV-2 infection was selective and not a consequence of global protein synthesis inhibition.

The lack of surface expression could be explained by either release from the cell surface or intracellular retention. Release of human Fas ligand from the surface has been shown to be mediated by a matrix metalloproteinase (19). However, a specific inhibitor of this enzyme, BB94 (19), did not prevent HSV-2 inhibition of surface expression (data not shown). Moreover, soluble Fas ligand was not observed in the supernatant of HSV-2-exposed KFL cells (data not shown).

To test the possibility that HSV-2 caused intracellular trapping of Fas ligand, we examined total cellular expression of Fas ligand in HSV-2-infected KFL cells that had been fixed and permeabilized (Fig. 5). Our findings indicate that HSV-2 did not inhibit total cellular levels of Fas ligand, although surface expression was markedly inhibited (67% inhibition). Thus, although Fas ligand was produced, HSV-2 infection prevented the protein from reaching the cell surface.

The role of the Fas apoptotic pathway as an antiviral mechanism. The inhibition of Fas ligand surface expression by HSV-2 suggests that the Fas-Fas ligand interaction plays a role in the antiviral defense mechanism against this virus. The effects of an intact cellular suicide pathway on viral titers were assessed. K562 cells were infected with 0.5 PFU of HSV-2 per cell and treated with either anti-Fas IgM or control IgM at 100 ng/ml. (It is important to note that K562 cells are not susceptible to Fas-mediated apoptosis unless they are infected with a virus [35a]). After 4 and 7 days, viral titers were measured (Table 1). Anti-Fas treatment significantly decreased levels of HSV-2 in the culture media. These data suggest that the Fas-Fas ligand pathway of apoptosis may act as an antiviral im-

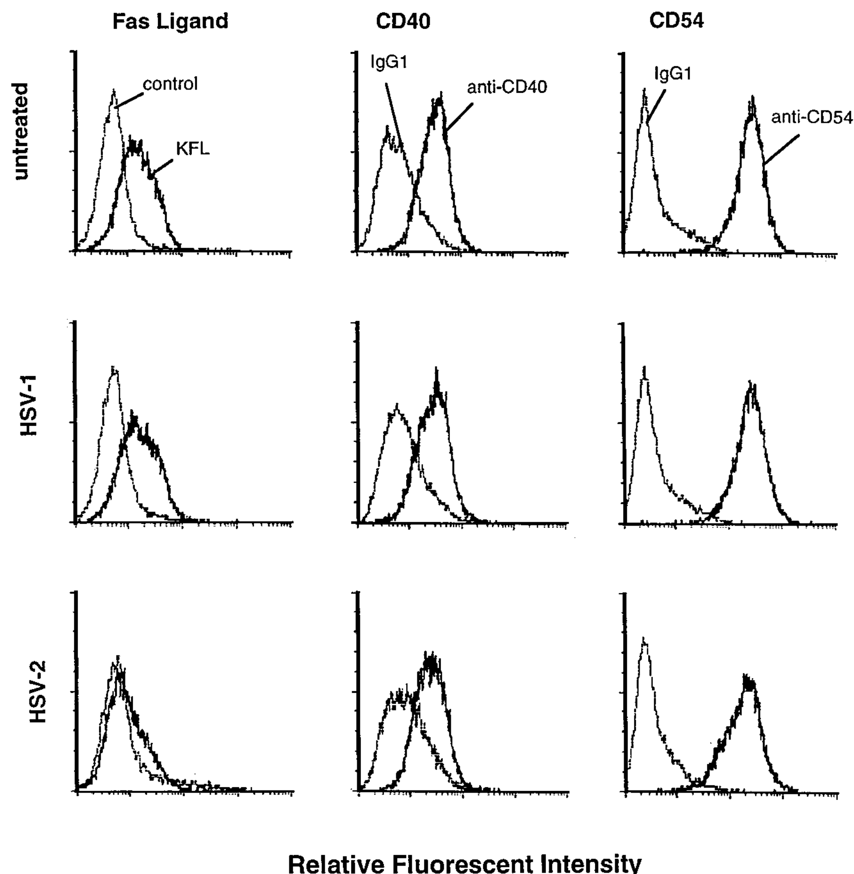

FIG. 4. Inhibition of Fas ligand cell surface expression by HSV-2. KFL cells were infected with either HSV-1 or HSV-2 at 3 PFU per cell and incubated for 3 days at 37°C. Cells were stained for Fas ligand surface expression. Control histograms in the left column represent staining of untransfected parental K562 cells. KFL cells were also stained for CD40 and CD54. Control histograms in the center and right columns represent staining of KFL cells with control IgG1 antibodies.

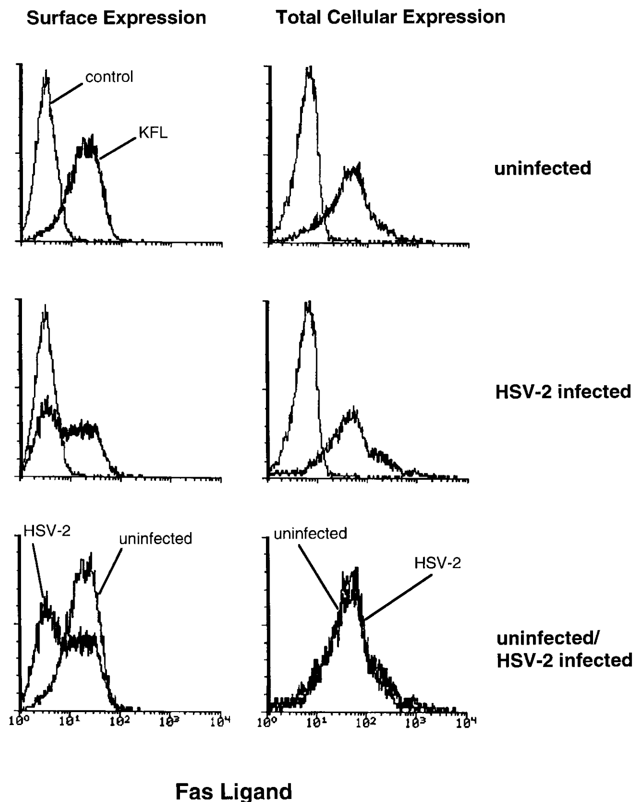

FIG. 5. Intracellular trapping of Fas ligand by HSV-2. KFL cells were infected with HSV-2 at 0.5 PFU per cell or left uninfected and incubated at 37°C for 4 days. Cells were stained for surface expression of Fas ligand (left column). Total cellular staining (right column) was performed after paraformaldehyde fixation and detergent permeabilization of the cells. Untransfected, parental K562 cells were used as negative controls. The histograms in the bottom panels of both the right and left columns demonstrate the overlay of Fas ligand staining histograms for uninfected and HSV-2-infected KFL cells.

mune mechanism; and consequently, the HSV-2-mediated inhibition of Fas ligand surface expression may have relevance to the pathogenesis of the infection.

DISCUSSION

Viruses modulate immunity in order to serve the needs of their own life cycles. For instance, tumor necrosis factor has been shown to possess a significant antiviral effect by selectively killing infected cells (27, 40) and adenovirus produces a specific protein that inhibits tumor necrosis factor-mediated cytolysis of infected cells, presumably thereby enhancing viral production (8, 13).

Fas ligand belongs to the tumor necrosis factor family of molecules (37). We have found that activation of the Fas apoptotic pathway has an antiviral effect for HSV-2 and that this virus inhibits the surface expression and consequently the activity of Fas ligand. The early death of an infected cell is an effective means of limiting viral replication. Our findings suggest that the capacity of HSV-2 to thwart programmed cell death of infected cells is an important mechanism for the virus to enhance its capacity to produce virus.

It is interesting that the defect in Fas ligand cell surface expression mediated by HSV-2 is not related to a defect in mRNA expression or to protein production but instead involves disruption in intracellular transport. This mechanism may be similar to the inhibition of surface MHC class I expression mediated by adenovirus (1, 2, 5, 17, 36) or HSV-1 (12, 16, 42). Thus, alterations in host proteins at an intracellular level may be an important mechanism for viruses to influence immunity.

HSV-2 inhibition of Fas ligand is likely to be involved in natural infections, since keratinocytes, a primary target of HSV-2 infection, are able to express Fas antigen in vivo in a number of clinical situations, including herpes zoster virus infection (28, 33, 41). Additionally, Fas ligand-expressing T cells migrating into herpesvirus lesions are potential targets for HSV-2-mediated Fas ligand inhibition, since activated T cells can be infected by HSV-2 (4, 20, 29, 32) and since we have shown that HSV-2 inhibits Fas ligand activity in these cells.

The data shown in this report suggest that Fas-Fas ligand interactions are important in HSV infections. Although both HSV-1 and HSV-2 have the capacity to induce Fas antigen expression in cells exposed to virus, only HSV-2 appears to be capable of regulating Fas ligand expression. HSV-1 and HSV-2 are closely related, with a high degree of sequence similarity; nevertheless, their clinical manifestations are distinct. HSV-2 infections are more severe than HSV-1 infections, and they have a greater tendency to recur (7, 22, 31, 39). We propose that the ability of HSV-2 to regulate Fas ligand activity may contribute to its greater propensity for recurrence and enhanced severity of infection in comparison with those characteristics of HSV-1.

We have recently postulated that Fas-mediated apoptosis may be a potent antiviral immune mechanism (35a). Many different viruses, including HSV-1 and HSV-2, rapidly enhanced the expression of Fas antigen, and treatment of productively infected cells with a Fas agonist markedly reduced the production of infectious virions. Inhibition of Fas ligand expression by HSV-2 may represent a potent mechanism for the virus to enhance viral replication in vivo.

TABLE 1. Anti-Fas-mediated inhibition of HSV-2 viral production

Immunoglobulin treatment[a]	Day of infection	HSV-2 viral titers (PFU/ml)	% Anti-Fas inhibition[b]
Control IgM	4	2.0×10^5	
Anti-Fas IgM	4	6.7×10^4	67
Control IgM	7	2.2×10^6	
Anti-Fas IgM	7	5.8×10^4	97

[a] K562 cells were infected with 0.5 PFU of HSV-2 per cell and treated with 100 ng of anti-Fas or control IgM per ml.

[b] Inhibition of viral titers mediated by anti-Fas IgM treatment of HSV-2-infected K562 cells compared with that after treatment with control IgM.

REFERENCES

1. **Andersson, M., S. Paabo, T. Nilsson, and P. Peterson.** 1985. Impaired transport of class I MHC antigens as a possible means for adenoviruses to evade immune surveillance. Cell **43:**215–222.
2. **Beier, D., J. Cox, D. Vining, P. Cresswell, and V. Engelhard.** 1994. Association of human class I MHC alleles with the adenovirus E3/19K protein. J. Immunol. **152:**3862–3872.
3. **Bertoletti, A., A. Sette, F. Chisari, A. Penna, M. Levrero, M. Carli, F. Fiaccadori, and C. Ferrari.** 1994. Natural variants of cytotoxic epitopes are T-cell receptor antagonists for antiviral cytotoxic T cells. Nature (London) **369:**407–410.
4. **Braun, R., H. Teute, H. Kirchner, and K. Munk.** 1984. Replication of herpes simplex virus in human T lymphocytes: characterization of the viral target cell. J. Immunol. **132:**914–919.
5. **Burgert, H., and S. Kvist.** 1985. An adenovirus type 2 glycoprotein blocks cell surface expression of human histocompatibility class I antigens. Cell **41:**987–997.

6. **Casali, P., G. Rice, and M. Oldstone.** 1984. Viruses disrupt functions of human lymphocytes. Effects of measles virus and influenza virus on lymphocyte-mediated killing and antibody production. J. Exp. Med. **159:**1322–1337.
7. **Corey, L., H. Adams, Z. Brown, and K. Holmes.** 1983. Genital herpes simplex virus infections: clinical manifestations, course, and complications. Ann. Intern. Med. **98:**958–972.
8. **Duerksen-Hughes, P., W. Wold, and L. Gooding.** 1989. Adenovirus E1A renders infected cells sensitive to cytolysis by tumor necrosis factor. J. Immunol. **143:**4193–4200.
9. **Engers, H., J. Louis, R. Zubler, and B. Hirt.** 1981. Inhibition of T cell-mediated functions by MVM(i), a parvovirus closely related to minute virus of mice. J. Immunol. **127:**2280–2285.
10. **Ettinger, R., D. Panka, J. Wang, B. Stanger, S.-T. Ju, and A. Marshak-Rothstein.** 1995. Fas ligand-mediated cytotoxicity is directly responsible for apoptosis of normal CD4+ T cells responding to a bacterial superantigen. J. Immunol. **154:**4302–4308.
11. **Frankel, N., E. Schirmer, L. Wyatt, G. Katsafanas, E. Roffman, R. Danovich, and C. June.** 1990. Isolation of a new herpes virus from CD4+ T cells. Proc. Natl. Acad. Sci. USA **87:**748–752.
12. **Fruh, K., K. Ahn, H. Djaballah, P. Sempe, P. van Endert, R. Tampe, P. Peterson, and Y. Yang.** 1995. A viral inhibitor of peptide transporters for antigen presentation. Nature (London) **375:**415–418.
13. **Gooding, L., L. Elmore, A. Tollefson, H. Brady, and W. Wold.** 1988. A 14,700 MW protein from the E3 region of adenovirus inhibits cytolysis by tumor necrosis factor. Cell **53:**341–346.
14. **Henkart, P.** 1994. Lymphocyte-mediated cytotoxicity: two pathways and multiple effector molecules. Immunity **1:**343–346.
15. **Hill, A., B. Barnett, A. McMichael, and D. McGeoch.** 1994. HLA class I molecules are not transported to the cell surface in cells infected with herpes simplex virus types 1 and 2. J. Immunol. **152:**2736–2741.
16. **Hill, A., P. Jugovic, I. York, G. Russ, J. Bennick, J. Yewdell, H. Ploegh, and D. Johnson.** 1995. Herpes simplex virus turns off the TAP to evade host immunity. Nature (London) **375:**411–415.
17. **Jefferies, W., and H. Burgert.** 1990. E3/19K from adenovirus 2 is an immunosubversive protein that binds to a structural motif regulating the intracellular transport of major histocompatibility complex class I proteins. J. Exp. Med. **172:**1653–1664.
18. **Kagi, D., F. Vignaux, B. Ledermann, K. Burki, V. Depraetere, S. Nagata, H. Hengartner, and P. Golstein.** 1994. Fas and perforin pathways as major mechanisms of T cell-mediated cytotoxicity. Science **265:**528–530.
19. **Kayagaki, N., A. Kawasaki, T. Ebata, H. Ohmoto, S. Ikeda, S. Inoue, K. Yoshino, K. Okumura, and H. Yagita.** 1995. Metalloproteinase-mediated release of human Fas ligand. J. Exp. Med. **182:**1777–1783.
20. **Kirchner, H., C. Kleinicke, and H. Northoff.** 1977. Replication of herpes simplex virus in human peripheral T lymphocytes. J. Gen. Virol. **37:**647–649.
21. **Klenerman, P., S. Rowland-Jones, S. McAdam, J. Edwards, S. Daenke, D. Lalloo, B. Koppe, W. Rosenberg, D. Boyd, A. Edwards, P. Giangrande, R. Phillips, and A. McMichael.** 1994. Cytotoxic T-cell activity antagonized by naturally occurring HIV-1 Gag variants. Nature (London) **369:**403–407.
22. **Lafferty, W., R. Coombs, J. Benedetti, C. Critchlow, and L. Corey.** 1987. Recurrences after oral and genital herpes simplex virus infection. Influence of site of infection and viral type. N. Engl. J. Med. **316:**1444–1449.
23. **Lowin, L., M. Hahne, C. Mattmann, and J. Tschopp.** 1994. Cytolytic T-cell cytotoxicity is mediated through perforin and Fas lytic pathways. Nature (London) **370:**650–653.
24. **Matzinger, P.** 1991. The JAM assay. A simple assay for DNA fragmentation and cell death. J. Immunol. Methods **145:**185–192.
25. **McChesney, M., and M. Oldstone.** 1987. Viruses perturb lymphocyte functions: selected principles characterizing virus-induced immunosuppression. Annu. Rev. Immunol. **5:**279–304.
26. **McKisic, M., F. Paturzo, D. Gaertner, R. Jacoby, and A. Smith.** 1995. A nonlethal rat parvovirus infection suppresses rat T lymphocyte effector functions. J. Immunol. **155:**3979–3986.
27. **Mestan, J., W. Digel, S. Mittnacht, H. Hillen, D. Blohm, A. Moller, H. Jacobsen, and H. Kerchner.** 1986. Antiviral effects of recombinant tumour necrosis factor in vitro. Nature (London) **323:**816–819.
28. **Oishi, M., K. Maeda, and S. Sugiyama.** 1994. Distribution of apoptosis-mediating Fas antigen in human skin and effects of anti-Fas monoclonal antibody on human epidermal keratinocyte and squamous cell carcinoma cell lines. Arch. Dermatol. Res. **286:**396–407.
29. **Posavad, C., J. Newton, and K. Rosenthal.** 1994. Infection and inhibition of human cytotoxic T lymphocytes by herpes simplex virus. J. Virol. **68:**4072–4074.
30. **Razvi, E., and R. Welsh.** 1995. Apoptosis in viral infections. Adv. Virus Res. **45:**1–60.
31. **Reeves, W., L. Corey, H. Adams, L. Vontver, and K. Holmes.** 1981. Risk of recurrence after first episodes of genital herpes. Relation to HSV type and antibody response. N. Engl. J. Med. **305:**315–319.
32. **Rinaldo, C., B. Richter, P. Black, R. Callery, L. Chess, and M. Hirsch.** 1979. Replication of herpes simplex virus and cytomegalovirus in human leukocytes. J. Immunol. **120:**130–135.
33. **Sayama, K., S. Yonehara, Y. Watanabe, and Y. Miki.** 1994. Expression of Fas antigen on keratinocytes in vivo and induction of apoptosis in cultured keratinocytes. J. Invest. Dermatol. **103:**330–334.
34. **Schirmer, E., L. Wyatt, K. Yamanishi, W. Rodriguez, and N. Frankel.** 1991. Differentiation between two distinct classes of viruses now classified as human herpesvirus 6. Proc. Natl. Acad. Sci. USA **88:**5922–5926.
35. **Sieg, S., L. Xia, Y. Huang, and D. Kaplan.** 1995. Specific inhibition of granzyme B by parainfluenza virus type 3. J. Virol. **69:**3538–3541.
35a. **Sieg, S., Y. Huang, and D. Kaplan.** Submitted for publication.
35b. **Sieg, S., Z. Yildirim, D. Smith, and D. Kaplan.** Submitted for publication.
36. **Signas, C., M. Katze, H. Persson, and L. Philipson.** 1982. An adenovirus glycoprotein binds heavy chains of class I transplantation antigens from man and mouse. Nature (London) **299:**175–178.
37. **Takahashi, T., M. Tanaka, J. Inazawa, T. Abe, T. Suda, and S. Nagata.** 1994. Human Fas ligand: gene structure, chromosomal location and species specificity. Int. Immunol. **6:**1567–1574.
38. **Vaux, D., G. Haecker, and A. Strasser.** 1994. An evolutionary perspective on apoptosis. Cell **76:**777–779.
39. **Whitley, R.** 1990. Herpes simplex viruses, p. 1843–1887. *In* B. Fields and D. Knipe (ed.), Fields virology. Raven Press, New York.
40. **Wong, G., and D. Goeddel.** 1986. Tumour necrosis factor α and β inhibit virus replication and synergize with interferons. Nature (London) **323:**819–822.
41. **Wrone-Smith, T., T. Johnson, B. Nelson, L. Boise, C. Thomspon, G. Nunez, and B. Nickoloff.** 1995. Discordant expression of bcl-x and bcl-2 by keratinocytes in vitro and psoriatic keratinocytes in vivo. Am. J. Pathol. **146:**1079–1088.
42. **York, I., C. Roop, D. Andrews, S. Riddell, F. Graham, and D. Johnson.** 1994. A cytosolic herpes simplex virus protein inhibits antigen presentation to CD8+ T lymphocytes. Cell **77:**525–535.