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Herpes simplex virus-infected cells disarm killer lymphocytes

(interleukin 2/Herpesvirus hominis/natural killer cells)

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ABSTRACT **Human endothelial cells or human foreskin fibroblasts infected with herpes simplex viruses (HSVs) potently inhibit the lytic activity of natural killer cells and interleukin 2-activated killer cells. The inhibition occurs after as little as 8 hr of viral infection and requires contact between effector cells and HSV-infected targets. Inhibition evidently stems from direct blockade of killer cell function because killer cells placed atop HSV-infected targets rapidly become incapable of lysing subsequently added HL-60 or K-562 cells. The impairment of killer cell function is prevented when protein glycosylation in HSV-infected cells is blocked with tunicamycin. These studies may be relevant for understanding the persistence of herpes simplex virus infections and, further, suggest a mechanism for failed immune surveillance.**

Natural killer cells (NK cells) are thought to eliminate abnormal cells that arise from parasitism by intracellular pathogens, particularly viruses, or from oncogenic stimuli causing malignant transformation (1–3). NK cells are normally present in the circulation and, along with other populations of lymphocytes, are further activated by the T-cell lymphokine interleukin 2 (IL-2) (4–9). These so-called lymphokine-activated killer cells, or LAK cells, display a greater lytic capacity and recognize a broader range of target cells than their nonactivated counterparts (6–9). Despite these presumably capable killer cells, humans are frequently afflicted with cancer and with persistent or recurrent viral infections.

A particularly relevant instance in which the immune system fails to eliminate a viral pathogen is that of human infection with herpes simplex virus (HSV). NK cells are necessary to control HSV in humans (10) but, nevertheless, fail to eradicate the infection. While investigating the role of killer lymphocytes in the recognition and elimination of HSV-infected cells, we made the unexpected observation that cytolytic activity of NK cells and LAK cells is remarkably suppressed following their contact with HSV-infected target cells. This down-regulation of cytotoxic effectors by cells infected with a common virus not only suggests a mechanism for persistence of HSV infections but also may serve as a model, in general, of failed immune surveillance.

METHODS

Cell Culture. Human foreskin fibroblasts were cultured in tissue culture medium (RPMI-1640 with 25 mM Hepes supplemented with 2 mM L-glutamine, 100 units of penicillin per ml, and 100 μg of streptomycin per ml) and 10% (vol/vol) fetal bovine serum. Human umbilical vein endothelium was prepared and cultured as described (11). K-562 and HL-60 cell lines were grown in suspension culture in the same medium as above. They were passaged as necessary to maintain the density below 10^6 cells per ml.

Virus Infection. HSV type 1 (HSV-1), strain 17 syn+, and HSV type 2 (HSV-2), strain HG52, were propagated and titrated in rabbit skin cells as described (12). Virus stock was prepared at 10^7 plaque-forming units (pfu)/ml of tissue culture medium with 5% calf serum and stored at −70°C in small aliquots. Confluent monolayers of target cells were infected with HSV by overlaying with 100 μl of virus stock per well and incubating for 1 hr at 35°C (multiplicity of infection ≈ 10 pfu per target cell). After the infecting incubation, the spent virus stock was removed and monolayers were overlaid with their original medium and incubated for various times before exposing them to killer cells.

In experiments comparing the effects of adenovirus, strain AD4, to those of HSV, A549 cells were used as targets (13, 14). Viral infection of A549 cells was carried out as above.

Killer Cells. NK activity was assayed in freshly isolated peripheral blood mononuclear cells. From 60 ml of sterile, heparinized venous blood, 3-ml samples were mixed with 5 ml of sterile phosphate-buffered saline and overlaid onto 3 ml of Histopaque-1077 (Sigma). The tubes were centrifuged at 400 × *g* for 30 min at room temperature; then the mononuclear cell layers were recovered and pooled. The resulting cell fraction was washed twice in phosphate-buffered saline and suspended in tissue culture medium. LAK cells were generated from peripheral blood mononuclear cells prepared from 42 ml of venous blood as above, but after the final wash the cells were suspended in tissue culture medium supplemented with 5% human serum and 1000 units of recombinant IL-2 per ml (Cetus). The cell concentration was adjusted to 10^6 per ml and the cells were incubated in a tissue culture incubator for 6 or 7 days.

An NK cell-enriched population of LAK cells was prepared by modification of the technique reported by Melder *et al.* (15). Briefly, peripheral blood mononuclear cells were isolated from 42 ml of venous blood and suspended at 10^7 per ml in tissue culture medium. Monocytes were depleted by adherence to plastic for 90 min at 37°C. The monocyte-depleted mononuclear cells were then suspended at 2×10^6 per ml in the above LAK cell medium and placed in 25-cm^2 culture flasks in a tissue culture incubator for 24 hr, after which the nonadherent cell fraction was suspended by rocking, removed, and set aside. The adherent cell layer was washed twice; the washes were discarded. The nonadherent cell fraction was then centrifuged and one-half of the resulting supernatant fluid was combined with an equal volume of fresh LAK cell medium. This conditioned medium was

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Abbreviations: HSV, herpes simplex virus; NK, natural killer; IL-2, interleukin 2; LAK, lymphokine-activated killer; E:T, effector:target; pfu, plaque-forming units.

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returned to the adherent cell layer, which was then incubated for an additional 7 days.

Cytotoxicity and Adherence. Cytotoxic activity of NK cells and LAK cells was determined essentially as described (16). Summarily, in 48-well tissue culture plates, confluent monolayers of fibroblasts or endothelial cells, pretreated as described for individual experiments, were labeled in a tissue culture incubator for 2 hr with ^{51}Cr (1.5–3 μCi per well; 1 Ci = 37 GBq). After labeling, each well was washed twice and then overlaid with 500 μl of tissue culture medium containing the effector cells (treatment wells) or no effector cells (spontaneous release wells). All data points were determined in triplicate or quadruplicate. Contact between effectors and targets was ensured by centrifugation at 50 × *g* for 3 min. Except as noted below, the plate was then incubated for 4 hr. Following incubation, the plate was centrifuged at 400 × *g* for 5 min so that insoluble radioactive material (e.g., detached cells) was pelleted. The radioactivity in the supernatant fluid and in the NaOH-solubilized pellet of each well was then determined by γ counting (Beckman). For each well, % release was calculated (supernatant counts/total counts) and the replicates were averaged. Specific lysis was then calculated as: (mean % release in treatment wells − mean % release in spontaneous wells)/(100% − mean % release in spontaneous wells). The standard deviation (SD) of each datum point was estimated from the partial derivatives of the specific lysis equation (17).

In some experiments, cytotoxicity was measured in sandwiches consisting of a monolayer of unlabeled, adherent target cells either infected or not with HSV. This adherent layer was then overlaid with effector cells and, subsequently, with a labeled target cell layer, either HL-60 cells or K-562 cells.

The adherence of effector cells to target cell monolayers was determined as described (16). In brief, effector cells were washed and then labeled for 2 hr at 37°C with 80–100 μCi of ^{51}Cr. The labeled cells were washed twice and suspended at the desired concentration. Confluent monolayers of fibroblasts or endothelial cells, pretreated as described for the particular experiment, were washed and overlaid with radiolabeled effector cells. Contact was ensured by centrifugation as above and the plate was incubated for 1 hr. The nonadherent cells were removed from each well by aspiration; the adherent cell layer was washed twice. Total radioactivity in the pooled nonadherent fractions and in the adherent fraction was determined as above, and the % nonadherence (representing nonadherent cells and spontaneously released radioactivity) was calculated for each well; replicates were then averaged. The % spontaneous release of ^{51}Cr from labeled effectors occurring over the course of the experiment was determined independently. Percent specific adherence was calculated as: 100 − (mean % nonadherence − mean % spontaneous release/100% − mean % spontaneous release).

Statistical Testing. Individual datum points were compared by Student's two-tailed *t* test applied to the mean and SD of replicate samples.

RESULTS

Human foreskin fibroblasts infected for 4 hr with HSV-1 are, as previously shown (1, 2), more readily lysed by NK cells than are noninfected controls (Fig. 1*A*). Surprisingly, however, as the viral infection progresses, this enhanced sensitivity to NK cell lysis is lost (Fig. 1*A*). This reversal is not an artifact of the more prolonged virus infection—that is, (*i*) target cells retain full viability for >48 hr following HSV-1 infection, (*ii*) ^{51}Cr, which is used in the cytotoxicity assay, is identically incorporated by virus-infected targets and normal control cells, and (*iii*) the spontaneous release of this radiolabel in the absence of NK cells is the same for infected and uninfected cells.

The defective killing of HSV-infected targets also obtains for LAK cells, which ordinarily are more efficient and recognize a broader range of target cells. In fact, LAK cells readily lyse cultured, uninfected human fibroblasts (Fig. 1*B*); however, LAK cell lysis of fibroblasts infected for 18 hr with HSV-1 is markedly curtailed (Fig. 1*B*, left). Furthermore, diminished destruction by LAK cells is not unique to HSV-1-infected fibroblasts but also occurs with infected human endothelial cells (Fig. 1*B*, right). Endothelial cells are likely to contact circulating killer cells *in vivo* and are known host cells in human HSV-1 infections (18, 19). As is true of

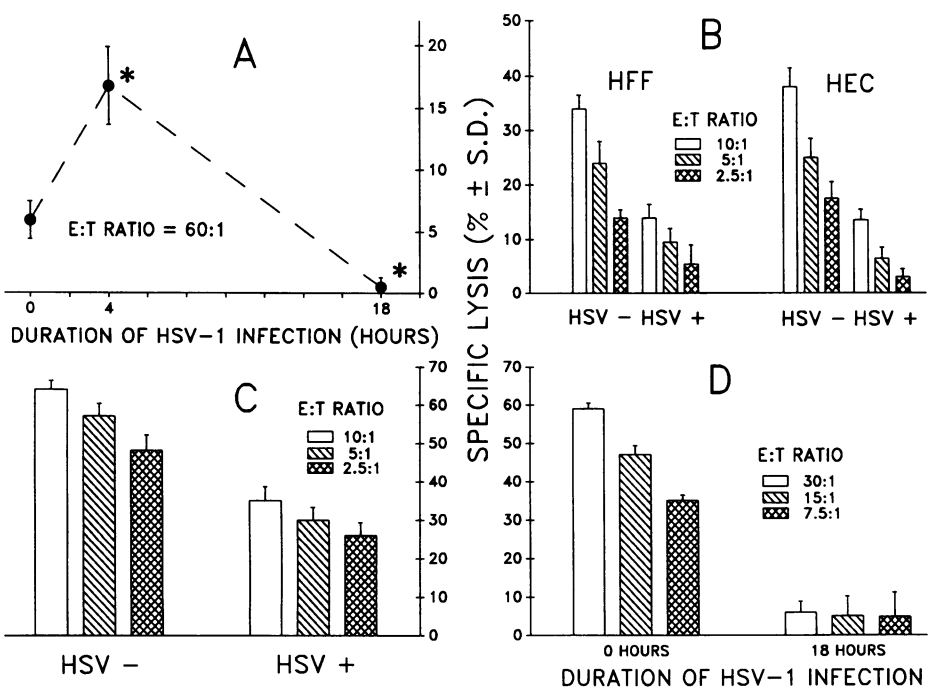

FIG. 1. Influence of HSV-1 infection on lysis of fibroblasts and endothelial cells by NK and LAK cells. (*A*) NK cell killing of HSV-1-infected fibroblasts as a function of the duration of HSV-1 infection. Similar data were obtained at lower effector to target (E:T) ratios. The asterisks indicate $P < 0.02$ compared to 0 hr. (*B*) Lysis of human foreskin fibroblasts (HFF) and human endothelial cells (HEC) by LAK cells: Influence of prolonged HSV-1 infection of target cells. Target cells were infected with HSV-1 for 18 hr (HSV +) or left alone (HSV −). Lysis of HSV + cells was significantly decreased in all cases. (*C*) Lysis of HL-60 cells by LAK cells in a sandwich with HSV-1-infected and normal fibroblasts. LAK cells were placed in contact with fibroblasts infected for 18 hr with HSV-1 (HSV +) or not (HSV −). Two hours later radiolabeled HL-60 cells were centrifuged onto each well. Specific lysis by LAK cells contacting HSV + substratal cells was significantly impaired ($P \leq 0.002$). (*D*) Lysis of fibroblasts by LAK cells generated from NK-enriched precursors. Lysis of virus-infected targets (18 hr) was significantly diminished ($P \leq 0.003$).

impaired killing by NK cells, diminished lysis by LAK cells is also dependent upon the duration of HSV-1 infection, unaltered in targets infected for only 4 hr but declining progressively as infection is prolonged. Thus, in a representative experiment, confluent endothelial cells were either unmanipulated (control) or infected with HSV-1 for 4, 8, or 18 hr before the addition of LAK cells at three E:T ratios. Specific lysis of quadruplicate samples was then determined. Compared to control, the mean decrement in lysis at 4 hr of infection was 0.0% ± 3.9%; at 8 hr, 20.0% ± 5.2%; and at 18 hr, 73% ± 3.9% ($P < 0.002$ at 8 and 18 hr by two-tailed t test).

The defective killing is evidently not due to sluggish onset of an effective lytic phase because even if the usual 4-hr chromium release assay is prolonged to 18 hr, no enhancement of cytolytic activity is observed. For example, endothelial cells in two culture plates prepared on the same day were either infected for 18 hr with HSV-1 or not. Both plates were then labeled with ^{51}Cr and overlaid with LAK cells at three E:T ratios. Specific lysis of quadruplicate samples was determined after 4 hr of killing in one plate and after 18 hr of killing in the second. Compared to the uninfected controls, the mean decrement in lysis of HSV-infected targets was the same after either 4 or 18 hr of killing (90% ± 3.6% vs. 89% ± 2.1%, respectively).

The decreased susceptibility to lysis by killer cells is not unique to HSV-1 or to the particular strain of HSV-1 examined—that is, diminished lysis also occurs with target cells infected by HSV-2 (Table 1). In contrast, infection with another common DNA virus, adenovirus, fails to protect against LAK cell lysis (Table 1).

The decrement in killer cell lytic activity is almost certainly a consequence of the viral infection of target cells and not an epiphenomenon due to a contaminating compound—e.g., γ-interferon (20)—in the virus preparation. Thus, prevention of the viral infection in target cells (by neutralizing antibody to HSV-1) or prior inactivation of the viral inoculum (by UV irradiation) completely averts the subsequent impairment of killer cell function. In the first instance, serial dilutions of human IgG, containing neutralizing antibody to HSV-1, were mixed with viable virus and added to target cells; subsequently, the antibody/virus inoculum was removed and the target cells were incubated for 18 hr. Under these conditions, morphologic evidence of viral infection and the decrement in LAK-mediated lysis were each inhibited in an antibody dose-dependent fashion (IC_{50} = 30 μg of IgG protein per ml). In the second instance, target cells were exposed to HSV-1 that had been inactivated by UV irradiation (pfu/ml following irradiation = 0) (21). Although such UV-inactivated viruses attach to target cells, their replication is deficient; concomitantly they were unable to provoke inhibition of LAK-mediated lysis.

The killing of target cells by NK and LAK cytotoxic effectors proceeds through a series of coordinated steps initiated by recognition and adhesion. In this regard, there is a very slight tendency for LAK cells to adhere less well to HSV-1-infected target cells than to normal targets (Table 2). In related experiments, however, cells from the LAK cell population that failed to adhere to 18-hr infected endothelium were overlaid on fresh, uninfected endothelial cells and examined for lytic activity. These nonadherent cells failed to lyse normally sensitive targets (experimental 7.4% ± 1.8% vs. control lysis 24% ± 4%; $P < 0.01$). These experiments suggest that selective nonadherence of particularly active cytotoxic effector cells is not an explanation for our results. This suggestion is further supported in the following experiments.

The failure of killer cells to lyse HSV-infected targets is not due to an innate resistance of the targets; rather, it evidently results from target cell-mediated inhibition of killer cell activity. This is clear from a series of sandwich experiments. For these, NK or LAK cells were first placed atop a monolayer of fibroblasts that had previously been infected, or not, with HSV-1. After a variable period of contact, the killer cell/target cell layer was then further overlaid with a fresh, radiolabeled target cell population; for LAK experiments this fresh target population was HL-60 cells; for NK cell experiments, it was K-562 cells. Each target was chosen for its known effector-cell sensitivity. Brief contact between LAK cells and HSV-1-infected fibroblasts inhibits LAK cell lysis of subsequently added HL-60 cells (Fig. 1*C*). A finite period of contact between the killer cells and HSV-infected targets is necessary before inhibition occurs; if LAK cells are placed atop HSV-infected fibroblasts and HL-60 cells are immediately added, lysis of HL-60 is not significantly impaired (not shown).

In analogous sandwich experiments, the ability of NK cells to lyse K-562 targets was similarly inhibited by contact with 18-hr HSV-1-infected fibroblasts. As expected, however, NK cells that contact uninfected fibroblasts, or those that contact fibroblasts infected with HSV-1 for only 4 hr, readily lyse K-562 cells. For example, in two experiments at an E:T ratio of 32, lysis of K-562 by NK cells exposed to uninfected fibroblasts was 54% ± 3.4% and 64% ± 1.8%. For NK cells on 4-hr HSV-1-infected fibroblasts, lysis of K-562 was identical (55% ± 2.8% and 65% ± 1.8%, respectively). In contrast, NK cells on 18-hr HSV-1-infected fibroblasts lysed only 33% ± 3.4% and 46% ± 1.3%, respectively. In each experiment, the values for 18-hr infection were significantly lower than uninfected or 4-hr infection ($P < 0.001$).

In further experiments with the sandwich model, we selectively radiolabeled either the infected, bottom (fibroblasts) layer or the uninfected, top (HL-60) layer and examined lysis by LAK effectors. These experiments (data not shown) confirmed that with 18-hr HSV-1 infection the decrease in target cell lysis was global—i.e., neither underlying nor overlying target cells were efficiently lysed by LAK cells exposed to HSV-1-infected fibroblasts.

Table 1. LAK cells lyse adenovirus-infected cells but not cells infected with HSV-1 or HSV-2

Exp.	Virus infection	Specific lysis, % 20 effectors per target	Specific lysis, % 10 effectors per target
1	None	66.6 ± 3.8	55.2 ± 1.9
	Adenovirus	76.3 ± 0.9	55.7 ± 0.9
	HSV-1	27.8 ± 6.0	21.2 ± 5.1
2	None	44.7 ± 1.7	28.0 ± 4.0
	HSV-2	13.8 ± 2.3	10.8 ± 4.2
	HSV-1	5.2 ± 1.4	1.5 ± 1.1

A549 cells (Exp. 1) or fibroblasts (Exp. 2) were left uninfected (None) or infected with virus for 18 hr. Values are expressed as mean ± SD.

Table 2. Adherence of LAK cells to normal and HSV-1-infected target cells

E:T ratio	Specific adherence, % Uninfected targets	*P*	Infected targets
10:1	60 ± 3.2	NS	50 ± 9.4
3.2:1	68 ± 2.5	0.02	55 ± 4.3
1:1	63 ± 0.8	NS	53 ± 6.5
0.32:1	62 ± 6.3	NS	52 ± 6.8

Endothelial target cells were infected, or not, with HSV-1 for 18 hr prior to addition of LAK cells. NS, not significant. Values are expressed as mean ± SD.

The inhibition of killer cell activity is not the result of interaction with viral particles or with soluble factors—that is, LAK cells directly preexposed to HSV-1 retain full lytic activity when subsequently placed on uninfected fibroblasts (Table 3, experiment 1). Furthermore, supernatant fluids obtained from HSV-1-infected targets and from infected targets cocultured with LAK cells do not affect LAK cell lysis of naive fibroblasts (Table 3, experiment 2). Prostaglandins, which may be very short lived in supernatant fluids, may inhibit NK cell cytolytic activity (22). However, blockade of prostaglandin production with indomethacin has no direct effect on LAK cell killing of normal targets nor does it restore LAK cell cytolysis of HSV-1-infected targets (Table 3, experiment 3). Finally, virus-infected cells apparently inhibit cytolytic activity of killer cells without assistance from accessory cells. We prepared a homogeneous population of LAK cells (15), >90% demonstrating a NK phenotype—CD-3 negative and CD-16 positive. These purified killer cells also failed to lyse HSV-1-infected targets (Fig. 1*D*).

Thus, the inhibition of killer cell activity is mediated by their direct contact with HSV-infected cells; we sought to determine whether a surface-expressed molecule on infected cells is responsible. Tunicamycin, an inhibitor of N-linked protein glycosylation, blocks the *de novo* synthesis of host cell and viral glycoproteins (23). We find that target cells infected with HSV-1 and then incubated for 18 hr in the presence of tunicamycin lose the capacity to inhibit LAK cell cytolysis (Table 4). This suggests that surface expression of glycoproteins is critically involved in suppressing killer cell lytic efficiency. As further confirmation, tunicamycin also fully restored lytic activity against HL-60 cells in sandwiches of LAK cells plus HSV-1-infected fibroblasts (not shown). Although viral proteins homologous, and potentially inhibitory, to IL-2 have been recently described (24, 25), the suppressive glycoproteins on HSV-infected cells are apparently unrelated—that is, direct addition of recombinant IL-2 (1000 units/ml) to the cytolysis assay modestly enhances LAK-cell-specific lysis of HSV-1-infected cells (from 1.6% ± 4.2% to 9.8% ± 4%, $P < 0.03$ by Student's two-tailed *t* test—but this increment is minimal, and lysis compared to uninfected controls (61% ± 5.3% and 68% ± 1.7% in the absence and presence of IL-2, respectively) remains profoundly impaired.

Table 4. Impaired LAK cell lysis is reversed by tunicamycin treatment of HSV-1-infected target cells

Tunicamycin, μg/ml	Specific lysis, %		
	Uninfected targets	*P*	Infected targets
0	72 ± 1.0	≪0.005	26 ± 8.3
0.5	74 ± 1.4	<0.005	60 ± 2.0
1	72 ± 1.8	0.07	60 ± 5.2
2	68 ± 5.0	NS	65 ± 3.6

Target cells were infected, or not, with HSV-1, overlaid with fresh culture medium containing tunicamycin, and incubated for 18 hr. The E:T ratio was 25:1. Similar results were obtained at other E:T ratios. NS, not significant. Values are expressed as mean ± SD.

DISCUSSION

HSVs cause incurable, lifelong human disease. Though sometimes trivial, HSV infections can produce extreme morbidity and death. To our knowledge, a compelling explanation for failure of the human immune system to eliminate these viruses has not been forthcoming. Our results may provide some insight, in that target cells infected with HSV-1 or HSV-2 inhibit the lytic activity of NK and LAK cells. This inhibition protects not only the virus-infected target but also uninfected targets as well. The degree of inhibition is dependent upon the duration of HSV-1 infection and only becomes manifest relatively late in its course. Contact between the effector and the target cell is required and, therefore, inhibition appears to be independent of numerous well-known soluble mediators, such as prostaglandins, interleukins, interferons, and tumor necrosis factors. Finally, the onset of inhibitory activity would seem to be contingent upon the synthesis and surface exhibition of new glycoproteins by the target cell.

Table 3. Effects of selected treatments upon LAK cell lysis of HSV-1-infected target cells

Exp.	Treatment	E:T ratio	Specific lysis, %	
			Uninfected targets	Infected targets
1*	HSV-exposed LAK cells	7:1	36 ± 2.2	0.3 ± 3.9
		3.5:1	28 ± 3.5	4.3 ± 5.9
	Control LAK cells	7:1	33 ± 1.5	3.1 ± 4.8
		3.5:1	22 ± 0.8	−0.2 ± 3.7
2†	Conditioned medium			
	HSV (+) LAK (−)	20:1	74 ± 1.2	—
	HSV (+) LAK (+)	20:1	75 ± 0.3	—
	HSV (−) LAK (+)	20:1	73 ± 1.4	—
	HSV (−) LAK (−)	20:1	71 ± 1.8	—
3‡	Indomethacin, μg/ml			
	50	30:1	78 ± 1.0	23 ± 1.6
	5	30:1	77 ± 0.4	26 ± 3.3
	0	30:1	78 ± 0.6	24 ± 1.9

HSV-1-infected targets were incubated for 18 hr prior to LAK cell exposure. Values are expressed as mean ± SD.

*LAK cells were exposed to HSV-1 at a multiplicity of infection of 10. After 1 hr, the cells were washed once, counted, and overlaid on target cells. Control LAK cells were mock-infected and handled in parallel. Similar data were obtained with LAK cells exposed to HSV-1 for 18 hr.

†Fibroblasts were infected for 18 hr with HSV-1 [HSV (+)] or mock-infected [HSV (−)]; then wells were washed and overlaid with fresh tissue culture medium [LAK (−)] or medium containing LAK cells [LAK (+)] adjusted to give an E:T ratio of 20:1. After 4 hr of incubation, the "conditioned medium" was removed, freed of cells by centrifugation, and overlaid at 75% (vol/vol) on fresh, radiolabeled fibroblasts with fresh LAK cells.

‡The usual cytolytic assays were conducted in the presence of indomethacin.

The observation that HSV-1-infected target cells can inhibit NK and LAK cell function might appear to conflict with previously published information (1, 2). Indeed, the ability of peripheral blood mononuclear cells to lyse HSV-1-infected fibroblasts has been a traditional measure of NK cell activity. Our results, however, do not contradict these earlier studies. We find that fibroblasts and endothelial cells briefly infected with HSV-1 are, in fact, lysed by peripheral blood mononuclear cells, whereas uninfected controls are not. It is only later in the course of HSV-1 infection (18 hr) that the inhibitory activity is seen (Fig. 1*A*). In simple NK cell assays, this relatively late onset of inhibition could mistakenly be attributed to the loss of a recognition molecule on the infected cells or to the general morbidity of the infected targets. Instead, our sandwich experiments show that prolongedly infected targets actively inhibit NK cell activity. This active inhibition is also demonstrated in our studies with LAK cells—that is, their cytolytic capacity toward targets, which they readily lyse prior to HSV-1 infection, progressively decreases following infection. Sandwich experiments with LAK cells further confirm active inhibition of these effectors.

In examining the mechanism for killer cell modulation, we have shown that adhesion of effector cells to prolongedly infected targets is slightly diminished (Table 2). This may play a role in diminished cytolysis of infected cells but does not explain the induction of cytolytic incapacity for subsequently added targets. Moreover, there is no evidence this diminished adhesion reflects selective nonadherence of active cytotoxic effectors—that is, cells from the original effector population that fail to adhere to HSV-1-infected targets are virtually incapable of lysing fresh, naive target cells. This impotence of effector function was confirmed in sandwich experiments in which NK and LAK cells when juxtaposed to HSV-1-infected targets become incapable of lysing a second, normally sensitive target cell. For significant inhibition to occur, however, some period of preexposure of effector cells to infected targets is required. This supports a hypothesis that intimate contact between effectors and infected targets is necessary for inhibitory signals to be effected.

Our results are reminiscent of prior studies by Heiskala *et al.* (26, 27). To review: these workers demonstrated that NK cells following contact with naturally occurring, NK-resistant targets would no longer efficiently lyse K-562 cells. This inhibition did not involve accessory mononuclear cells and was independent of soluble factors. Onset of cytolysis inhibition was very slow, minimally detectable after 3 hr of contact between NK cells and NK-resistant targets and <50% decreased after 20 hr of contact. Those results are qualitatively similar to our own studies; however, we find that HSV-1-infected targets inhibit NK cell cytotoxicity more rapidly and to a greater degree.

Perhaps more relevant to the present studies are recent reports that NK cell function is inhibited by peptides corresponding to portions of transmembrane retroviral glycoproteins (28, 29). For instance, a 17-amino acid peptide, corresponding to a highly conserved region in the envelope proteins of murine leukemia virus and feline leukemia virus and in the transmembrane glycoproteins of human T-cell lymphotrophic viruses I and II, was shown to inhibit NK cell lysis of K-562 cells (28). In a more recent study, two peptides, corresponding to regions of the gp41 transmembrane glycoprotein of human immunodeficiency virus, were also shown to inhibit NK cell cytolytic activity (29). When NK cells incubated with the inhibitory peptides were then treated for several hours with recombinant IL-2, lytic activity increased somewhat but was still deficient compared to control effectors.

The seeming paradox has not escaped our attention that HSV-1-infected targets, which are ultimately doomed to destruction by their infection, develop the capacity to inhibit NK and LAK cell cytolytic function. Perhaps expression of this phenomenon during natural herpesvirus infections occurs under conditions that are less paradoxical and more critical for maintenance of the virus. It remains to be shown however whether killer cell inhibition actually occurs during natural HSV infections and what the consequences of such inhibition might be.

Finally, taken together, our results and those of others may imply existence of a signal transduction pathway in NK cells and IL-2-activated killer cells whose activation inhibits lytic activity. Confirmation that such an inhibitory pathway exists would provide insights into killer cell regulation and, perhaps, suggest a mechanism for failure of immune surveillance.

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