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Murine Cellular Cytotoxicity to Syngeneic and Xenogeneic Herpes Simplex Virus-Infected Cells

STEVE KOHL,[1]* DAVID B. DRATH,[2] AND LIAN S. LOO[1]

Department of Pediatrics and Program of Infectious Diseases[1] and Department of Surgery,[2] University of Texas Medical School, Houston, Texas 77025

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Cellular cytotoxicity of C57BL/6 adult mice peritoneal cells to xenogeneic (Chang liver) and syngeneic (BL/6-WT3) herpes simplex virus (HSV)-infected cells was analyzed in a 6-h ^{51}Cr release assay. There was no difference in antibody-dependent cellular cytotoxicity to either target. There was no natural killer cytotoxicity to targets with cells from uninfected mice except at very high effector cell ratios. HSV-infected (2×10^4 PFU intraperitoneally 1 day previously) mice mediated significantly higher antibody-dependent cellular cytotoxicity and required less antibody (10^{-5} versus 10^{-2} dilution), fewer cells, and less time to kill than cells from uninfected mice. HSV-infected mice mediated natural killer cytotoxicity but preferentially killed syngeneic HSV-infected cells. Stimulation of cytotoxicity was not virus specific since influenza-infected mice mediated similar levels of cytotoxicity to HSV-infected targets. There was no difference in morphology (95% macrophage) or in the percentage of FcR-positive cells, but infected mice had more peritoneal cells and generated higher levels of superoxide in response to opsonized zymosan or phorbolmyristate acetate. These data demonstrate nonspecific virus-stimulated metabolic and effector cell function which may enhance clearance of virus in an infected host.

Cellular cytotoxicity to herpes simplex virus (HSV)-infected cells has been extensively analyzed in vitro (2, 3, 5, 6, 8–10, 12–17, 19–23, 25–36, 38–45; S. Kohl, J. Infect. Dis., in press). Due to *H-2* restriction, all successful murine or human systems reported to date have utilized syngeneic or autologous cells to analyze T cell cytotoxicity (5, 19, 23, 26, 34, 38–40; K. A. Storthz, J. W. Smith, and L. A. Wilson, Fed. Proc. **41**:571, 1982). In contrast, only one report of murine natural killer cytotoxicity (NKC) (27) and no reports of antibody-dependent cellular cytotoxicity (ADCC) have utilized syngeneic or autologous target cells. Indeed, it has been suggested to these authors that antiviral ADCC may require xenogeneic target cells and therefore is of limited in vivo significance.

For these reasons we now report the results of a series of experiments successfully delineating ADCC to syngeneic murine HSV-infected target cells. In addition, we have compared the ADCC and NKC of normal and HSV-infected mice to syngeneic and xenogeneic HSV-infected and uninfected target cells. Leukocytes from infected mice produced increased amounts of superoxide and were able to mediate both NKC and ADCC in a more efficient manner than those of uninfected mice.

MATERIALS AND METHODS

Virus. HSV type 1 (HSV-1, HE strain, originally isolated in the laboratory of A. J. Nahmias, Emory University, Atlanta, Ga., from a neonate with disseminated HSV infection) was propagated in Chang liver cells grown in Earle minimal essential medium (GIBCO Laboratories, Grand Island, N.Y.) supplemented with 10% heat-inactivated fetal calf serum (Flow Laboratories, Rockville, Md.), penicillin (100 U/ml), and streptomycin (100 μg/ml) (supplemented minimal essential medium; SMEM). Where specified, HSV-1 was harvested in SMEM without heat-inactivated fetal calf serum. The virus had a titer of 10^6 PFU per ml on Chang liver monolayers under a 1.5% methylcellulose (Fisher Scientific Co., Fairlawn, N.J.) overlay. We previously reported that the survival rate of 18- to 22-g male C57BL/6 mice inoculated with 10^6 PFU of HSV-1 HE strain was 84% (14, 15).

Influenza virus used was influenza A/Hong Kong/68 H_3N_2, kindly provided by Philip Wyde, Baylor College of Medicine, Houston, Tex. This was passage nine in C3H mice of a mouse-adopted influenza virus and contained 6.2 50% tissue culture infective dose and 4.2 50% lethal dose per ml.

Target cells. Monolayers of Chang liver cells, which are xenogeneic to C57BL/6 mice, were infected with HSV-1 (HE strain) at a multiplicity of infection of 0.5 to 1, 18 h before use, trypsinized, labeled with radioactive sodium chromate the day of use, and suspended to a final concentration of 10^5 cells/ml in SMEM as

described (12–17). Uninfected Chang liver cells were prepared in a similar fashion.

BL/6WT-3 cells (C57BL/6 mouse embryo fibroblasts transformed by simian virus 40) which are syngeneic for C57BL/6 mice were kindly provided by S. S. Tevethia, Pennsylvania State University College of Medicine, Hershey, Pa. These cells were utilized essentially as previously described by Carter et al. (5). Confluent monolayers were infected with HSV-1 (multiplicity of infection, 0.5 to 1) for 1 h, after which time 100 μCi of ^{51}Cr was added. The cells were then incubated for 18 h, trypsinized, and washed once before suspension to a final concentration of 10^5 cells/ml in SMEM. Uninfected BL/6WT-3 cells were similarly prepared.

Animals. C57BL/6 adult (8 to 12 weeks old) mice were obtained from Timco Breeding Co., Houston, Tex. Animals infected with HSV-1 were inoculated intraperitoneally with 0.2 ml of a 1:10 dilution (2×10^4 PFU) of HSV in SMEM without heat-inactivated fetal calf serum. Resident peritoneal cells were collected as previously described and washed three times in SMEM before use in the microcytotoxicity assay (12, 14, 15).

Human effector cells. Human peripheral blood mononuclear cells were obtained as previously described from heparinized blood by sequential dextran sedimentation and Ficoll-Hypaque (Pharmacia Fine Chemical Co., Piscataway, N.J.) buoyant density centrifugation. Mononuclear cells were washed in Hanks balanced salt solution (GIBCO) four times before suspension in SMEM and use in the cytotoxicity assay (13, 16).

High-affinity leukocyte Fc receptor analysis. High-affinity Fc receptor analysis was performed as previously described (16) by using sheep red blood cells (Dutchland Laboratories, Denver, Pa.) sensitized with rabbit anti-sheep red cell antibody (a 1:256 dilution; Cappel Laboratory, Cochraneville, Pa., lot 14953). The percentage of peritoneal cells rosetting with three or more red blood cells of 200 counted cells was calculated.

Peritoneal cell morphology. Differential counts of peritoneal cells were performed on Wright-stained, Cytocentrifuge (Cytospin-Shandon, Southberry Products, Ltd., Runcorn, Cheshire, England)-prepared slides. One hundred cells were counted for each differential cell count.

Serum. Human immune serum consisted of a pool of sera prepared from four individuals with past HSV infections and HSV antibody titers determined by neutralization and ADCC (17). The neutralizing antibody titer of the pool was 1:32 and the ADCC titer with human effector cells was 10^{-5}. Nonimmune serum consisted of pooled sera from four individuals with a negative clinical history of previous HSV infection and no detectable HSV-neutralizing or ADCC antibodies. All serum was heat inactivated at 56°C for 30 min and stored at −20°C.

Microcytotoxicity assay. The microcytotoxicity assay was performed in triplicate in rigid polystyrene U-bottom well microtiter plates (Dynatech Laboratories, Inc., Alexandria, Va.) as previously described (12–17). A 100-μl portion of effector cells diluted to obtain the desired effector-to-target cell ratio, 50 μl of target cells (5×10^3), and 50 μl of HSV immune or nonimmune serum were added to each well. Before incubation, the microtiter plates were centrifuged for 5 min at $50 \times g$ to increase cell-to-cell contact in the assay. The covered plates were incubated at 37°C in a humidified atmosphere of 95% air and 5% CO_2 for 6 h unless otherwise specified.

To determine the amount of ^{51}Cr released from the target cells, we aspirated 100 μl from the top of each well without disturbing the cell button. To each well, 100 μl of 1 M NaOH was then added, and the total volume was aspirated into a separate container. All samples were counted in a Beckman 4000 gamma counter for 1 min. Chromium release was calculated according to the following formula: percent ^{51}Cr released = $(2A/A + B) \times 100$, where A equals counts per minute in the top 100 μl and B equals counts per minute in the bottom 100 μl to which NaOH was added. The standard deviation of triplicate assays was less than 10%.

NKC was defined as:

$$\% \text{ NKC} = \frac{(\%^{51}\text{Cr release of target cells plus effector cells}) - (\%^{51}\text{Cr release of target cells})}{100 - (\%^{51}\text{Cr release of target cells})} \times 100$$

ADCC was defined as:

$$\% \text{ ADCC} = \frac{\begin{array}{c}(\%^{51}\text{Cr release of target cells plus effector cells plus immune serum}) - \\ (\%^{51}\text{Cr release of target cells plus effector cells plus nonimmune serum})\end{array}}{\begin{array}{c}100 - (\%^{51}\text{Cr release of target cells plus effector cells plus nonimmune serum})\end{array}} \times 100$$

Superoxide production by peritoneal cells. Superoxide release was measured as previously described (7). Briefly, either monolayers prepared and treated as described by Michell and co-workers (21) or suspensions of cells at a final concentration of 10^6 were incubated in the presence of 80 μM ferricytochrome *c* and Hanks balanced salt solution containing 0.1% glucose. Appropriate monolayers contained either opsonized zymosan (10 particles per cell) or phorbolmyristate acetate (PMA, 0.5 μg/ml). Monolayers with and without superoxide dismutase (150 U) were incubated at 37°C for 30 min, after which time their supernatants were removed and centrifuged. Cytochrome *c* reduction was measured spectrophotometrically at 550 nm in a Beckman Du 8 spectrophotometer with a millimolar extinction coefficient of 19. In those experiments where cell suspensions were used, initial rate determinations were made on samples in the presence and absence of superoxide dismutase. The reactions were initiated by the addition of PMA to cuvettes in a thermostatically controlled cuvette chamber.

Statistical method. Analysis of differences between mean values of paired results was performed by Student's two-tailed paired *t* test. Data is expressed as the mean ± standard error of the mean (SEM) of experiments.

TABLE 1. NKC of peritoneal cells from HSV-infected and uninfected mice to infected and uninfected target cells[a]

Target cells[b]	Mean % NKC ± SEM of peritoneal cells from:[c]		P[d]
	Control	HSV infected	
HSV-infected BL/6WT-3 (8)	5.3 ± 1.9	40.3 ± 6.8	<0.001
Uninfected BL/6WT-3 (7)	2.9 ± 1.9	26.3 ± 3.4	<0.001
HSV-infected Chang liver cells (6)	3.3 ± 2.2	22.2 ± 4.6	<0.05
Uninfected Chang liver cells (4)	4.2 ± 2.3	29.8 ± 1.7	<0.001

[a] The cytotoxicity assay was performed with an effector-to-target cell ratio of 100:1 and a 6-h incubation period.

[b] Cells were infected 18 h before assay with HSV-1. Numbers in parentheses are the number of separate experiments performed.

[c] Effector cells were from uninfected (control) or HSV-infected (2×10^4 PFU intraperitoneally 24 h before assay) adult C57BL/6 mice.

[d] P value is the significance of the difference between percent NKC for infected and control mouse peritoneal cells.

RESULTS

Target cell leakage. Spontaneous ^{51}Cr leakage of HSV-infected Chang liver cells was 17.2 ± 1.4% and 23.6 ± 1.8% after 6 and 18 h of incubation, respectively. The leakage of uninfected Chang liver cells was 22.1 ± 2.4% and 45.9 ± 0.8% after 6 and 18 h of incubation, respectively. These data are as previously reported (17). The leakage of the HSV-infected BL/6WT-3 cells was 55.1 ± 2.6% and 65.3 ± 11.1% after 6 and 18 h of incubation, respectively. The spontaneous leakage of the uninfected BL/6WT-3 cells was 43.8 ± 4.7% and 62.3 ± 12.7% after 6 and 18 h of incubation, respectively. Due to the very high ^{51}Cr release of BL/6WT-3 cells after 18 h of incubation, experiments with BL/6WT-3 cells were terminated after 6 to 7 h of incubation, whereas selected experiments utilizing Chang liver cells were carried out for 18 h.

NKC to HSV-infected and uninfected cells by peritoneal cells from HSV-infected and uninfected mice. The NKC to infected and uninfected target cells by peritoneal cells from infected and uninfected mice was examined by using an effector-to-target cell ratio of 100:1 in a 6-h assay (Table 1). There was uniformly low NKC with cells from control mice against any target cell utilized in a 6-h assay. Peritoneal cells from mice infected 1 day previously with HSV had significantly higher NKC to all target cells than did those from uninfected mice. NKC against BL/6WT-3 cells infected with HSV (40.3 ± 6.8%) was significantly higher than that against uninfected BL/6WT-3 (26.3 ± 3.4%, $P < 0.05$) or HSV-infected Chang liver cells (22.2 ± 4.6, $P < 0.01$). There was no significant difference in NKC against HSV-infected compared with uninfected Chang liver cells.

ADCC mediated by peritoneal cells from HSV-infected and uninfected mice to syngeneic and xenogeneic target cells. ADCC was analyzed in a 6-h assay with an effector-to-target cell ratio of 100:1 and a serum dilution of 1:20 (Table 2). There was no ADCC to uninfected target cells (data not shown). There was no significant difference in ADCC mediated by the peritoneal cells to HSV-infected BL/6WT-3 or Chang liver target cells. As seen with NKC, ADCC of peritoneal cells from infected mice was significantly ($P < 0.01$) higher than that of uninfected mice to both syngeneic (BL/6WT-3) and xenogeneic (Chang liver) cells.

Effect of varying effector-to-target cell ratios on cytotoxicity. To verify that the data presented in Tables 1 and 2 were not peculiar to the effector-to-target cell ratio utilized, experiments with effector-to-target cell ratios of 10:1 to 200:1 were examined (Fig. 1 through 3). NKC was not mediated by peritoneal cells from uninfected mice to uninfected target cells at any cell ratio (Fig. 1). Peritoneal cells from infected mice

TABLE 2. ADCC of peritoneal cells from HSV-infected and uninfected mice to syngeneic and xenogeneic HSV-infected target cells[a]

Target cells[b]	Mean % ADCC ± SEM of peritoneal cells from:[c]		P[d]
	Control	HSV infected	
HSV-infected BL/6WT-3 (8)	15.0 ± 4.5	33.1 ± 4.4	<0.01
HSV-infected Chang liver cells (5)	9.3 ± 3.4	30.3 ± 3.9	<0.01

[a] The cytotoxicity assay was performed with an effector-to-target cell ratio of 100:1, a serum dilution of 1:20, and a 6-h incubation period.

[b] The target cells were infected 18 h before assay with HSV-1. Numbers in parentheses are the number of separate experiments performed.

[c] Effector cells were from uninfected (control) or HSV-infected (2×10^4 PFU intraperitoneally, 24 h before assay) adult C57BL/6 mice.

[d] P value is the significance of the difference between percent ADCC for infected and control mouse peritoneal cells.

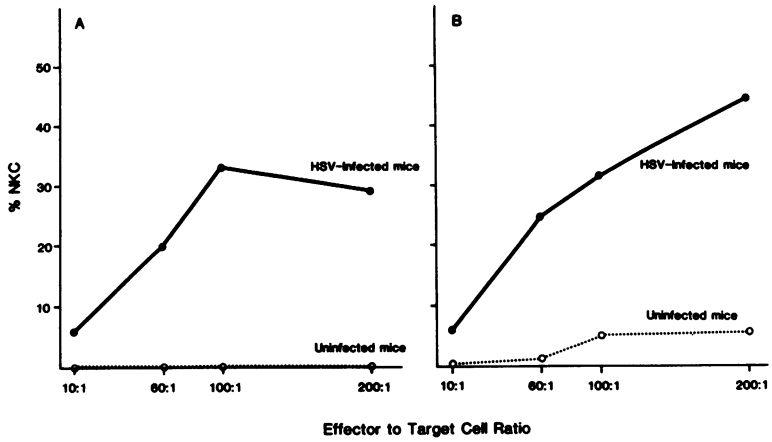

FIG. 1. NKC to uninfected target cells. Uninfected BL/6WT-3 cells (A) and Chang liver cells (B) were utilized in a 6-h cytotoxicity assay with peritoneal cells from uninfected (○) or HSV-infected (2×10^4 PFU HSV 24 h previously) (●) mice.

mediated NKC to uninfected target cells beginning at effector-to-target cell ratios of 60:1 and increasing at ratios of 100:1 (BL/6WT-3) or 200:1 (Chang liver cells) (Fig. 1). NKC was mediated by leukocytes from infected and uninfected mice to HSV-infected BL/6WT-3 cells (Fig. 2). NKC of peritoneal cells from infected mice was evident at effector-to-target cell ratios as low as 10:1 and reached a plateau value at a ratio of 100:1. NKC from uninfected mice was evident only at high effector-to-target cell ratios (100:1, 200:1) (Fig. 2A). NKC to HSV-infected Chang liver cells was only mediated by peritoneal cells from infected mice and was evident at effector ratios of 60:1 and reached a plateau value at effector-to-target cell ratios of 100:1 (Fig. 2B). As presented in Table 1, NKC was highest to HSV-infected BL/6WT-3 cells at any effector-to-target cell ratio utilized (Fig. 1 and 2). NKC to infected or uninfected Chang liver cells was of similar magnitude at all effector-to-target cell ratios (Table 1, Fig. 1B and 2B).

ADCC to HSV-infected BL/6WT-3 cells mediated by peritoneal cells from infected mice was higher than that by uninfected mice until very high effector-to-target cell ratios (200:1) were utilized. At that ratio the peritoneal cells from infected mice had low ADCC (Fig. 3A). ADCC

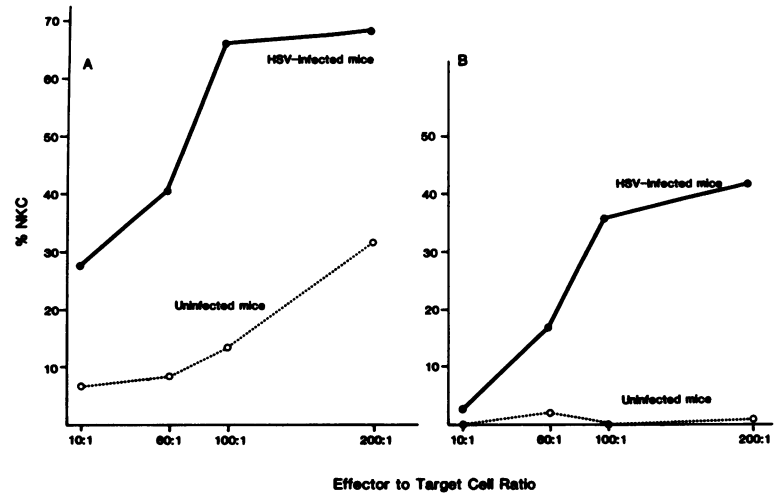

FIG. 2. NKC to HSV-infected target cells. HSV-infected BL/6WT-3 cells (A) and Chang liver cells (B) were utilized in a 6-h cytotoxicity assay with peritoneal cells from uninfected (○) or HSV-infected (●) mice.

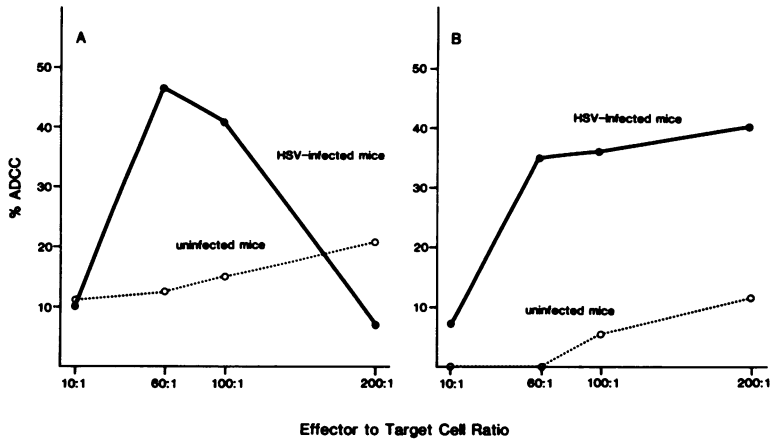

FIG. 3. ADCC to HSV-infected target cells. HSV-infected BL/6WT-3 cells (A) and Chang liver cells (B) were utilized in a 6-h cytotoxicity assay with peritoneal cells from uninfected (○) or HSV-infected (●) mice. The immune serum dilution was 1:20.

to HSV-infected Chang liver cells was more efficient by peritoneal cells from HSV-infected mice than from uninfected mice at all effector-to-target cell ratios tested (Fig. 3B).

Effect of antibody concentration on ADCC of peritoneal cells from HSV-infected and uninfected mice against syngeneic and xenogeneic target cells. The previous experiments were performed with a final immune serum dilution of 1:20. The effect on ADCC of varying the serum concentration from 10^{-1} to 10^{-6} was analyzed (Fig. 4). ADCC to HSV-infected BL/6WT-3 mediated by peritoneal cells from infected mice was higher than that of uninfected mice at all serum concentrations. Although no ADCC was detected beyond a serum concentration of 10^{-2} by using cells from uninfected mice, cells from infected mice still mediated significant ADCC in the presence of a serum concentration as low as 10^{-5} (Fig. 4A). The peritoneal cells from infected mice similarly were more active than those from uninfected mice against HSV-infected Chang liver cells. They required more antibody to mediate significant ADCC activity to Chang liver cells (10^{-1} for cells from uninfected mice and 10^{-2} for cells from HSV-infected mice, Fig. 4B) than to BL/6WT-3 cells (Fig. 4A).

Effect of varying the incubation time on cellular cytotoxicity. The kinetics of NKC and ADCC were analyzed by terminating the experiments

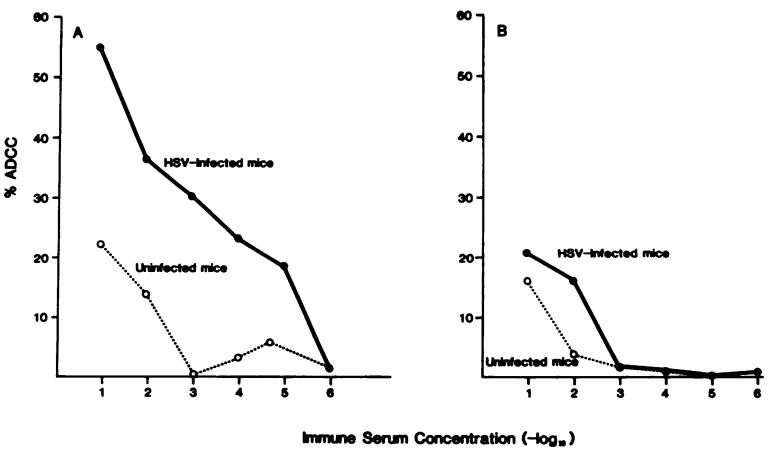

FIG. 4. Effect of various concentrations of immune serum on ADCC. HSV-infected BL/6WT-3 (A) or Chang liver (B) cells were utilized in a 6-h cytotoxicity assay with peritoneal cells from uninfected (○) or HSV-infected (●) mice at an effector-to-target cell ratio of 100:1.

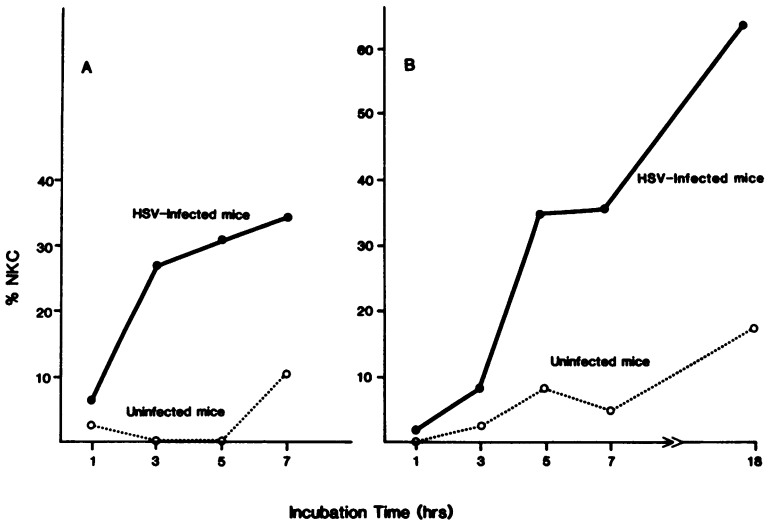

FIG. 5. Kinetics of NKC to uninfected cells. Uninfected BL/6WT-3 (A) or Chang liver (B) cells were utilized in a cytotoxicity assay with peritoneal cells from uninfected (○) or HSV-infected mice (●) at an effector-to-target cell ratio of 100:1.

from 1 to 7 h after the initiation of the assay in the case of BL/6WT-3 cells and from 1 to 18 h in the case of Chang liver cells (see Fig. 7). As presented in Table 1, there was no significant NKC to any target cell mediated by peritoneal cells from uninfected mice in less than 7 h (Fig. 5 and 6). After 18 h of incubation, as previously reported (12, 14, 15), NKC was mediated by cells from uninfected mice to HSV-infected and uninfected Chang liver cells (Fig. 5B, 6B). The levels of NKC to infected and uninfected Chang liver cells were similar, as previously reported in the Chang liver system with human effector cells (13). NKC mediated by cells from HSV-infected mice was higher than that of control animals at all times after 1 h of incubation (Fig. 5 and 6). As presented in Table 1, NKC was highest to HSV-infected BL/6WT-3 cells at all times tested (Fig. 6A).

ADCC was more rapidly manifested by cells from infected mice than those from uninfected mice to both Chang liver and BL/6WT-3 infected

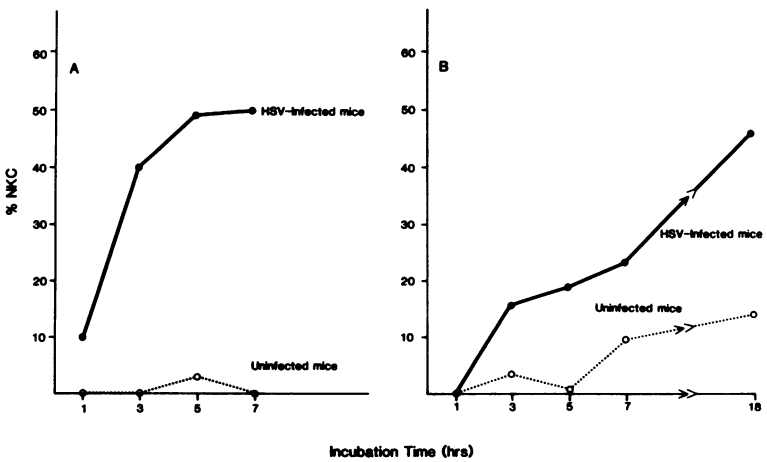

FIG. 6. Kinetics of NKC to HSV-infected cells. HSV-infected BL/6WT-3 (A) or Chang liver (B) cells were utilized in a cytotoxicity assay with peritoneal cells from uninfected (○) or HSV-infected (●) mice at an effector-to-target cell ratio of 100:1.

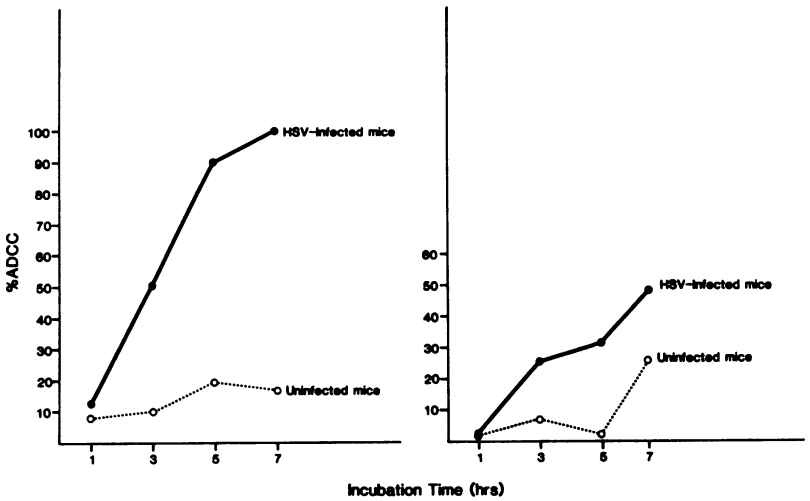

FIG. 7. The kinetics of ADCC to HSV-infected cells. HSV-infected BL/6WT-3 (A) or Chang liver (B) cells were utilized in a cytotoxicity assay with peritoneal cells from uninfected (○) or HSV-infected (●) mice at an effector-to-target cell ratio of 100:1. The immune serum dilution was 1:20.

cells. ADCC activity was readily detected by 3 h of incubation by using cells from infected animals compared with 5 to 7 h of incubation by using cells from uninfected mice (Fig. 7).

Time course of HSV-stimulated cytotoxicity. To determine the duration of stimulation of antiviral cytotoxicity caused by viral infection, mice were sacrificed 1 to 7 days after intraperitoneal inoculation with HSV and their peritoneal cells assayed in the cytotoxicity assay (Table 3). ADCC levels were maximum 1 day after infection and approached normal ADCC values 3 days postinfection. NKC was similarly maximum 1 day after infection but unlike ADCC remained elevated 7 days postinfection.

Cellular cytotoxicity of animals infected with HSV or influenza virus. To determine whether the increases in ADCC and NKC mediated by cells from HSV-infected mice were virus specific, experiments were performed with influenza virus-infected mice (Table 4). As seen with HSV infection, mice infected with influenza 1 day previously had increased ADCC to BL/6WT-3 and Chang liver HSV-infected cells and increased NKC to infected BL/6WT-3 cells. Unlike experiments with HSV, there was no increase in NKC to HSV-infected Chang liver cells. Increased cytotoxicity in influenza-infected mice in general was of a lower magnitude than seen with HSV. Nevertheless, these data demonstrate that increased cytotoxicity to HSV-infected cells was not virus specific.

Peritoneal cell enumeration and characterization. The effect of intraperitoneal infection 1 day previously with 2×10^4 PFU of HSV in C57BL/6 mice on the number and type of peritoneal cells was examined. As previously reported (14), there were significantly ($P < 0.001$) more peritoneal cells recovered from infected ($16.9 \times 10^6 \pm 2.3 \times 10^6$ cells per mouse, mean ± SEM of 11 experiments) than from uninfected ($4.4 \times 10^6 \pm 0.6 \times 10^6$ cells per mouse) animals. Table 5 lists the types of cells recovered from these animals. There was no difference in the cell differential count (95% monocyte macrophage and 5% lymphocyte) or the percentage of cells manifesting high-affinity immunoglobulin Fc receptors (6 to 8%) between uninfected and infected mice. Quantitation of virus present in the peritoneal cavity of mice 1 day after infection revealed approximately 0.004 PFU per peritoneal cell (5×10^5 PFU per mouse).

Superoxide production by peritoneal cells.

TABLE 3. Time course of HSV-stimulated cytotoxicity

Days after virus inoculation[a]	Cytotoxicity	
	% ADCC	% NKC
1	29.0	40.5
2	18.3	37.8
3	14.1	32.8
7	14.1	32.1
No virus (control)	13.6	0

[a] Days after intraperitoneal inoculation with 2×10^4 PFU of HSV.

[b] Murine peritoneal cells at an effector-to-target cell ratio of 100:1; a 1:20 serum dilution in a 6-h assay.

TABLE 4. Cellular cytotoxicity of peritoneal cells from uninfected mice and mice infected with HSV or influenza[a]

Peritoneal cells	Cytotoxicity[b]			
	% ADCC ± SEM to:		% NKC ± SEM to:	
	BL/6WT-3	Chang liver	BL/6WT-3	Chang liver
Uninfected	9.2 ± 8.2	0 ± 0	12.5 ± 1.8	3.1 ± 2.4
HSV infected[c]	37.2 ± 3.1	39.5 ± 14	89.9 ± 6.2	28.3 ± 1.8
Influenza infected[d]	23.3 ± 4.3	22.6 ± 2.3	36.4 ± 9.8	5.1 ± 0.1

[a] The cytotoxicity assay was performed at an effector-to-target cell ratio of 100:1, a serum dilution of 1:20 and a 6-h incubation period. The data are for two experiments.
[b] Target cells were BL/6WT-3 cells infected with HSV or Chang liver cells infected with HSV.
[c] Cells were from mice infected 1 day previously with HSV (2×10^4 PFU intraperitoneally).
[d] Cells were from mice infected 1 day previously with influenza virus (1.2 50% tissue culture infective dose).

Resting or nonstimulated superoxide release was routinely observed to be less than 0.5 nmol per 30 min per 10^6 cells in peritoneal cells from either uninfected or infected mice. Peritoneal cells responded to a stimulus such as PMA or opsonized zymosan, however, by markedly increasing their release of superoxide into the extracellular medium. The cells obtained from HSV-infected mice released greater than 2.5-fold more superoxide than cells from controls (Table 6). This result was consistent regardless of the stimulus used to trigger superoxide release. The rate of superoxide release after the addition of PMA over an 8-min time span was 0.37 and 0.62 nmol/min for control and infected cells, respectively.

DISCUSSION

We have demonstrated murine ADCC to HSV-infected syngeneic cells for the first time. An important technique that allowed for the consistent manifestation of mouse peritoneal cell ADCC by using target cells unsuitable for an overnight assay, due to high spontaneous ^{51}Cr leakage, was the low-speed centrifugation of the reaction mixture (target cells, effector cells, and antibody) before incubation. This presumably increased early cell-cell contact and accelerated the ADCC reaction (Table 2, Fig. 3).

Comparison of murine ADCC against HSV-infected syngeneic BL/6WT-3 target cells and xenogeneic Chang liver target cells revealed no major differences (Table 2). The syngeneic system was more sensitive to small amounts of antibody (Fig. 4) and displayed decreased ADCC when high effector-to-target cell ratios (200:1) of cells from HSV-infected mice (Fig. 3A) were used. The reason for this is not yet clear. It is possible that there is an *H-2* target-restricted suppressor cell function operating at high effector cell densities.

ADCC to syngeneic and xenogeneic cells mediated by cells from HSV-infected mice was higher at all effector ratios tested when compared with cells from uninfected mice (Table 2, Fig. 3). The cells from infected mice also required considerably less antibody to mediate ADCC (Fig. 4) and mediated ADCC more rapidly (Fig. 7) than did cells from uninfected mice. The increase in murine ADCC was not virus specific, since cells from influenza infected animals also had increased ADCC effector cell activity (Table 4).

Although there was no difference in the morphological cell types (Table 5), being 95% monocyte macrophages, or the percentage of cells with high-affinity immunoglobulin Fc receptors (6 to 8%) in HSV-infected and uninfected mice, infected mice had markedly more peritoneal cells and the cells displayed a higher metabolic activity as measured by superoxide release upon phagocytosis of opsonized zymosan or PMA stimulation (Table 6). These results differ from our previous experiments describing lower ADCC in infected mice (14). The probable explanation for this was the use of 100 times less virus in these experiments.

TABLE 5. Peritoneal cell characterization[a]

Characteristic	Peritoneal cells from:	
	Uninfected mice	HSV-infected mice[b]
% Lymphocytes[c]	5.1 ± 1.4	5.1 ± 1.2
% Monocyte macrophages[c]	94.9 ± 1.4	94.8 ± 1.2
% FcR positive[d]	6.2 ± 0.4	8.7 ± 1.4

[a] Results are the mean ± SEM for six experiments.
[b] Infected 1 day before assay with HSV (2×10^4 PFU intraperitoneally).
[c] Determined by morphology.
[d] Cells positive for high-affinity immunoglobulin G Fc receptors by the erythrocyte antibody rosette technique.

TABLE 6. Superoxide release

Stimulus	Superoxide release[a] by peritoneal cells from:		P[c]
	Uninfected mice	HSV-infected mice[b]	
PMA (0.5 μg/ml)	5.97 ± 1.65	16.70 ± 4.03	<0.05
Opsonized zymosan (10 particles per cell)	5.84 ± 1.98	16.78 ± 3.74	<0.05

[a] Results are mean ± SEM of four experiments and are expressed as nanomoles of cytochrome *c* reduced per 30 minutes per monolayer of 10^6 cells.

[b] Mice infected 1 day previously with HSV (2×10^4 PFU intraperitoneally).

[c] Significance of difference between superoxide releases of cells from uninfected and HSV-infected mice.

Although suppression of cellular immune function by viral infection is a well-known phenomenon (for review see reference 46), stimulation of phagocytosis by HSV (1) and peritoneal cell activation in mice by murine cytomegalovirus and other viruses (4, 37) has been recently reported. Similar early stimulation of ADCC effector function has been recently documented in a human with acute primary HSV infection (S. Kohl, J. Infect. Dis., in press). The obvious biological benefits of enhanced ADCC effector function, more rapid kill and a need for fewer cells and less antibody, is probably of particular importance early in a primary viral infection (Table 3). At this time antibody is just becoming available, and other antiviral mechanisms, such as T cell cytotoxicity, are not yet manifested.

The mechanism of, and possible link between, the increased ADCC and metabolic activity is under investigation. There is controversy regarding the role of the metabolic burst of phagocytic cells and the mechanism of ADCC (11, 18, 24, 43; C. F. Nathan, Fed. Proc. **41**:2206–2211, 1982). In a human in vitro system, interferon can stimulate macrophage ADCC (R. Reves, and S. Kohl, manuscript in preparation). It is reasonable to expect that interferons or other virus-stimulated lymphokines are likely candidates to explain increased in vivo ADCC function in mice as well. It also remains possible that viruses can directly stimulate effector function or mobilize pools of effector cells from other body compartments to increase local effector cell number and activity.

Murine NKC mediated by peritoneal cells from uninfected mice to syngeneic or xenogeneic infected or uninfected cells was uniformly low in a 6-h assay (Table 1). By using very high effector-to-target cell ratios (200:1) NKC was mediated to infected syngeneic cells (Fig. 2A). As previously described, NKC to HSV-infected Chang liver cells was detected in an 18-h assay (14, 15). HSV infection, as also previously described by us and others (2, 3, 14, 15, 27) markedly increased NKC to all target cells at all times and effector cell ratios analyzed (Table 1, Fig. 1, 2, 5 and 6). NKC was highest to HSV-infected BL/6WT-3 cells. This was not virus specific since the BL/6WT-3 cells infected with HSV were also preferentially lysed by murine cells from influenza-infected animals (Table 4). Increased murine NKC activity was not solely due to increased fragility of these cells as may be inferred from their high spontaneous chromium leakage. There was no preference of murine ADCC to BL/6WT-3 as compared with Chang liver HSV-infected cells (Tables 2 through 4).

Recent reports have similarly demonstrated preferential lysis of HSV-infected syngeneic target cells by rabbit effector cells (K. A. Storthz, J. W. Smith, and L. A. Wilson, Fed. Proc. **41**:571, 1982), and of HSV-infected syngeneic or allogeneic murine cells by spleen cells from HSV or vaccinia-infected mice (27). This has been suggested to represent augmentation of NKC by species-specific interferon production by infected target cells (27). More finite identification of the effector cell in this early, non-antibody-mediated cytotoxicity is ongoing.

As recently reported, work with human antiviral cellular cytotoxicity (S. Kohl and C. M. Moore, Immunology, in press), the results of an assay with syngeneic target cells in a murine system confirm previous results with Chang liver cells. Minor discrepancies in NKC activity to syngeneic cells versus xenogeneic or allogeneic cells may make their inclusion in NKC studies of some importance. The less favorable target cell chromium leakage characteristics would argue against their use in ADCC systems.

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LITERATURE CITED

1. **Armerding, D., P. Mayer, M. Scriba, A. Hren, and H. Rossiter.** 1981. In-vivo modulation of macrophage functions by herpes simplex virus type 2 in resistant and sensitive inbred mouse strains. Immunobiology **160**:217–227.
2. **Armerding, D., and H. Rossiter.** 1981. Induction of natural killer cells by herpes-simplex virus type 2 in resistant and sensitive inbred mouse strains. Immunobiology **158**:369–379.
3. **Armerding, D., M. M. Simon, U. Hammerling, G. J. Hammerling, and H. Rossiter.** 1981. Function, target cell preference, and cell-surface characteristics of herpes-simplex virus type-2-induced non-antigen-specific killer cells. Immunobiology **158**:347–368.
4. **Blanden, R. V., and C. A. Mims.** 1973. Macrophage acti-

vation in mice infected with ectromelia or lymphocytic choriomeningitis viruses. Aust. J. Exp. Biol. Med. Sci. 51:393–398.
5. **Carter, V. C., P. A. Schaffer, and S. S. Tevethia.** 1981. The involvement of herpes simplex virus type 1 glycoproteins in cell mediated immunity. J. Immunol. 126:1655–1660.
6. **Ching, C., and C. Lopez.** 1979. Natural killing of herpes simplex virus type 1-infected target cells: normal human responses and influence of antiviral antibody. Infect. Immun. 26:49–56.
7. **Drath, D. B., and M. L. Karnovsky.** 1975. Superoxide production by phagocytic leukocytes. J. Exp. Med. 141:257–262.
8. **Droller, M., P. Perlmann, and M. U. Schneider.** 1978. Enchancement of natural and antibody-dependent lymphocyte cytotoxicity by drugs which inhibit prostaglandin production by tumor target cells. Cell. Immunol. 39:154–164.
9. **Grewal, A. S., B. T. Rouse, and L. A. Babiuk.** 1977. Mechanisms of resistance to herpes viruses: comparison of the effectiveness of different cell types in mediating antibody-dependent cell-mediated cytotoxicity. Infect. Immun. 15:698–703.
10. **Heron, I., A. Moller-Larsen, and K. Berg.** 1977. Effector cell involved in cell-mediated cytotoxicity to cells infected with herpes simplex virus type 1. Infect. Immun. 16:48–53.
11. **Klassen, D. K., and A. L. Sagone.** 1980. Evidence for both oxygen and non-oxygen dependent mechanisms of antibody sensitized target cell lysis by human monocytes. Blood 56:985.
12. **Kohl, S., D. L. Cahall, D. L. Walters, and V. Schaffner.** 1979. Murine antibody dependent cellular cytotoxicity to herpes simplex virus infected target cells. J. Immunol. 123:25–30.
13. **Kohl, S., J. J. Frazier, S. B. Greenberg, L. K. Pickering, and L. S. Loo.** 1981. Interferon induction of natural killer cytotoxicity in human neonates. J. Pediatr. 98:379–384.
14. **Kohl, S., M. J. P. Lawman, B. T. Rouse, and D. L. Cahall.** 1981. The effect of herpes simplex virus infection on murine antibody-dependent cellular cytotoxicity and natural killer cytotoxicity. Infect. Immun. 31:704–711.
15. **Kohl, S., and L. S. Loo.** 1980. Ontogeny of murine cellular cytotoxicity to herpes simplex virus-infected cells. Infect. Immun. 30:847–850.
16. **Kohl, S., L. K. Pickering, T. G. Cleary, K. D. Steimetz, and L. S. Loo.** 1980. Human colostral cytotoxicity II. Relative defects in colostral leukocyte cytotoxicity and inhibition of peripheral blood leukocyte cytotoxicity by colostrum. J. Infect. Dis. 142:884–891.
17. **Kohl, S., S. E. Starr, J. M. Oleske, S. L. Shore, R. B. Ashman, and A. J. Nahmias.** 1977. Human monocyte-macrophage-mediated antibody-dependent cytotoxicity to herpes simplex virus infected cells. J. Immunol. 118:729–735.
18. **Koller, C. A., and A. F. LoBuglio.** 1981. Monocyte-mediated antibody-dependent cell-mediated cytotoxicity: the role of the metabolic burst. Blood 58:293–299.
19. **Lawman, M. J. P., B. T. Rouse, R. J. Courtney, and R. D. Walker.** 1980. Cell-mediated immunity against herpes simplex induction of cytotoxic T lymphocytes. Infect. Immun. 27:133–139.
20. **Melewicz, F. M., S. L. Shore, E. W. Ades, and D. J. Phillips.** 1977. The mononuclear cell in human blood which mediates antibody-dependent cellular cytotoxicity to virus-infected target cells. II. Identification as a K cell. J. Immunol. 118:567–573.
21. **Michell, R. H., S. J. Pancake, J. Noseworthy, and M. L. Karnovsky.** 1969. Measurements of rates of phagocytosis. The use of cellular monolayers. J. Cell Biol. 40:216–224.
22. **Moller-Larsen, A., I. Heron, and S. Haahr.** 1977. Cell mediated cytotoxicity to herpes-infected cells in humans: dependence on antibodies. Infect. Immun. 16:43–47.
23. **Nash, A. A., J. Phelan, and P. Wildy.** 1981. Cell-mediated immunity in herpes simplex virus-infected mice: H-2 mapping of the delayed-type hypersensitivity response and the antiviral T cell response. J. Immunol. 126:1260–1262.
24. **Nathan, C., and Z. Cohn.** 1980. Role of oxygen-dependent mechanisms in antibody induced lysis of tumor cells by activated macrophages. J. Exp. Med. 152:198–208.
25. **Oleske, J. M., R. B. Ashman, S. Kohl, S. L. Shore, S. E. Starr, R. Wood, and A. J. Nahmias.** 1977. Human polymorphonuclear leukocytes as mediators of antibody-dependent cellular cytotoxicity to herpes simplex virus-infected cells. Clin. Exper. Immunol. 27:446–453.
26. **Pfizenmaier, K., H. Jung, A. Starzinski-Powitz, M. Rollinghoff, and H. Wagner.** 1977. The role of T cells in anti herpes simplex virus immunity. Induction of antigen specific cytotoxic T lymphocytes. J. Immunol. 119:939–944.
27. **Plontek, G. E., R. Weltzin, and W. A. F. Tompkins.** 1980. Enhanced cytotoxicity of mouse natural killer cells for vaccinia and herpes virus-infected targets. RES J. Reticuloendothel. Soc. 27:175–188.
28. **Rager-Zisman, B., and A. C. Allison.** 1980. The role of natural killer (NK) cells in resistance to herpes simplex virus 1 (HSV-1) infection. Parasite Immunol. 2:71–84.
29. **Rager-Zisman, B., and B. R. Bloom.** 1974. Immunological destruction of herpes simplex virus I infected cells. Nature (London) 251:542–543.
30. **Ramshaw, I. A.** 1975. Lysis of herpesvirus-infected target cells by immune spleen cells. Infect. Immun. 11:767–769.
31. **Reichman, R. C., R. Dolin, M. M. Vincent, and A. S. Fauci.** 1977. Cell-mediated cytotoxicity to recurrent herpes simplex virus infections in man. Proc. Soc. Exper. Biol. Med. 155:571–576.
32. **Rodda, S. J., and D. O. White.** 1976. Cytotoxic macrophages: a rapid nonspecific response to viral infection. J. Immunol. 117:2067–2072.
33. **Rola-Pleszcynski, M.** 1981. In vitro induction of human cell-mediated cytotoxicity directed against herpes simplex virus-infected cells. Kinetics in normal donor and patients with recurrent herpes labialis. J. Clin. Lab Immunol. 6:39–43.
34. **Rouse, B. T., and M. J. P. Lawman.** 1980. Induction of cytotoxic T lymphocytes against herpes simplex virus type 1, role of accessory cells and amplifying factors. J. Immunol. 124:2341–2346.
35. **Rouse, B. T., R. C. Wardley, and L. A. Babiuk.** 1976. Antibody-dependent cell-mediated cytotoxicity in cows: a comparison of effector cell activity against heterologous erythrocytes and herpesvirus-infected bovine target cells. Infect. Immun. 13:1433–1441.
36. **Russell, A. S., J. S. Percy, and T. Kovithavongs.** 1975. Cell-mediated immunity to herpes simplex in humans: lymphocyte cytotoxicity measured by 51Cr release from infected cells. Infect. Immun. 11:355–359.
37. **Schleupner, C. J., G. A. Olsen, and L. A. Glasgow.** 1979. Activation of reticuloendothelial cells following infection with murine cytomegalovirus. J. Infect. Dis. 139:641–652.
38. **Sethi, K. K., and H. Brandis.** 1978. Interferon enhances T cell mediated cytotoxicity of H-2 compatible target cells infected with UV-inactivated herpes simplex virus. Arch Virol. 57:177–183.
39. **Sethi, K. K., I. Stroehmann, and H. Brandis.** 1980. Human T-cell cultures from virus-sensitized donors can mediate virus-specific and HLA-restricted cell lysis. Nature (London) 286:718–720.
40. **Sethi, K. K., and M. H. Wolff.** 1980. The nature of host-cell herpes-simplex virus interaction(s) that render cells susceptible to virus-specific cytotoxic T cells. Immunobiology 157:365–378.
41. **Shore, S. L., C. M. Black, and F. M. Melewicz.** 1976. Antibody-dependent cell mediated cytotoxicity to target cells infected with type 1 and type 2 herpes simplex virus. J. Immunol. 116:194–201.
42. **Shore, S. L., A. J. Nahmias, S. E. Starr, P. A. Wood, and D. E. McFarlin.** 1974. Detection of cell-dependent cytotoxic antibody to cells infected with herpes simplex virus.

Nature (London) **251**:350–352.
43. **Siebens, H., S. S. Tevethia, and B. M. Babior.** 1979. Neutrophil-mediated antibody-dependent killing of herpes-simplex-virus-infected cells. Blood **54**:88–94.
44. **Stanwek, T. L., D. E. Campbell, and A. J. Nahmias.** 1980. Spontaneous cytotoxicity mediated by human monocyte-macrophages against human fibroblasts infected with herpes simplex virus-augmentation by interferon. Cell. Immunol. **54**:413–416.
45. **Trofatter, K. F., Jr., and C. A. Daniels.** 1979. Interaction of human cells with prostaglandins and cyclic AMP modulators. I. Effects on complement-mediated lysis and antibody-dependent cell-mediated cytolysis of herpes simplex virus-infected human fibroblasts. J. Immunol. **122**:1363–1370.
46. **Wilkinson, P. C.** 1980. Leukocyte locomotion and chemotaxis: effects of bacteria and virus. Rev. Infect. Dis. **2**:293–318.