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Natural Killing of Herpes Simplex Virus Type 1-Infected Target Cells: Normal Human Responses and Influence of Antiviral Antibody

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Studies of a mouse model of genetic resistance to herpes simplex virus type 1 (HSV-1) indicate that the marrow-dependent effector cell of allogeneic resistance plays an important role in natural resistance to this virus infection. Since the marrow-dependent effector cell appears to be closely related to the natural killer (NK) cells, an NK assay with HSV-1-infected fibroblasts [NK(HSV-1)] has been developed to study this resistance mechanism in humans. Incubation of effector and target cells for 12 to 14 h gave the greatest percent specific release (%SR) and kept spontaneous ^{51}Cr release from infected target cells below 35%. Patients with Bruton's agammaglobulinemia demonstrated significant kill indicating antiviral antibody was not necessary. Seropositive individuals gave a 9% greater %SR than seronegative individuals. Depletion of B-cells consistently diminished NK(HSV-1) for seropositive individuals and augmented kill for seronegative individuals. Although antiviral antibody produced in culture may contribute to NK(HSV-1), depletion of B-cells allowed quantitation of NK(HSV-1) to the exclusion of most of the antibody-dependent kill. The NK cells detected by this assay showed many of the properties reported for NK cells with K562 targets. Two patients with severe herpesvirus infections demonstrated NK(HSV-1) responses greater than 2 standard deviations below the normal mean. Since normal individuals with virus infections have higher rather than lower natural kill, the low NK(HSV-1) may reflect their susceptibility to the virus infection.

Patients with certain primary immunodeficiency disorders (35), many newborn infants (22), and patients treated aggressively with chemo- and radiotherapy for cancer (1) or organ transplantation (16, 24, 34) often suffer herpesvirus infections of markedly increased severity. Since these patients usually have a depressed cell-mediated immune capacity (18, 35), the latter is thought to play a major role in host defense against herpesvirus infections (19). Humoral immune responses, on the other hand, are often normal or slightly exaggerated in these patients (19). The cell-mediated immune response is complex, however, and the specific mechanisms responsible for host defense against herpesvirus infections are not known. For example, even though the thymus-derived cell (T cell) plays a central role in the cell-mediated immune response, severe herpesvirus infections have only rarely been associated with specific T-cell deficiencies (36).

Because of recent observations made in this laboratory with a mouse model of genetic resistance (14), we have proposed that the marrow-dependent cell, which mediates allogeneic resistance (6), also plays an important role in defense against herpesvirus infections (15, 16). Recent studies with ^{89}Sr-treated mice indicate that, as was found with resistance to allogeneic marrow grafts (2), resistance to herpes simplex virus type 1 (HSV-1) in the mouse is a marrow-dependent function (16). Haller and Wigzell (8) reported that treatment of mice with ^{89}Sr also depressed markedly the natural killer (NK) cell function, suggesting a probable relationship between marrow-dependent cells and NK cells. Since assays of NK cell function might reflect marrow-dependent cell function as well (5), we have developed an NK assay utilizing HSV-1-infected target cells to determine whether NK cell (HSV-1) function reflects resistance to HSV-1 in humans. Described herein is an NK assay with HSV-1-infected fibroblasts [NK(HSV-1)] and the results obtained in the study of individuals with and without antiviral antibody. Also included are results obtained from the study of cord blood lymphocytes and effector cells from two patients with severe herpesvirus infections, patients in

which low NK(HSV-1) in vitro appeared to correlate with increased susceptibility to severe HSV-1 infections.

MATERIALS AND METHODS

Blood donors. Healthy adult laboratory personnel (males and females) volunteered for studies of NK cytotoxicity. Serum-neutralizing antibody titers and lymphocyte transformation studies, performed as described earlier (17), were utilized to determine whether individuals were seropositive or seronegative. In addition, sera from the seronegative donors used in these studies were evaluated for their capacity to mediate antibody-dependent cell-mediated cytotoxicity (ADCC). Addition of their heat-inactivated sera, at a final dilution of 1:100, failed to augment ^{51}Cr release with HSV-1-infected target cells, whereas sera from seropositive individuals consistently augmented lysis by 20 to 50%. Cord blood samples were obtained at delivery of healthy infants. Blood samples from patients with X-linked agammaglobulinemia (Bruton) were kindly provided by F. Siegal, S. Gupta, E. M. Smithwick, and R. A. Good. In addition, blood samples from patients with severe herpesvirus infections were provided by B. Park and S. Fikrig.

Effector cells. Mononuclear cells from heparinized venous blood were separated by the Ficoll-Hypaque density gradient sedimentation method of Böyum (4). Interface cells were washed three times in Ca^{2+}- and Mg^{2+}-free Hanks balanced salt solution and resuspended in RPMI 1640 medium containing 10% heat-inactivated fetal bovine serum (RPMI-FBS), 100 U of penicillin per ml, 100 μg of streptomycin per ml, 2 mM glutamine, and 20 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid). The cell concentration was adjusted to 5×10^6 cells per ml.

Target cells. Monolayer cultures of HSV-1-infected and uninfected human skin fibroblast (FS_4) cells were used as target cells. The FS_4 cells, kindly provided by Ed Havell, New York University, New York, N. Y., were cultured as monolayers in Dulbecco modified minimum essential medium supplemented with 10% FBS (DMEM-FBS), penicillin, and streptomycin. Monolayer cultures were infected with HSV-1 (2931 strain; 22) at a multiplicity of infection of about 5 plaque-forming units per cell at 37°C for 2 h. The infected FS_4 cells were rinsed and then removed with 0.025% trypsin in 0.02% ethylenediaminetetraacetic acid. For labeling, 2×10^6 to 3×10^6 cells in 0.2 ml of RPMI-FBS were incubated with 150 μCi of Na_2–^{51}Cr–O_4 (specific activity, 5 mCi/ml; New England Nuclear Corp., Boston, Mass.) for 1 h at 37°C with gentle agitation every 15 min. The labeling efficiency was approximately 14 to 17% and yielded 5 to 10 cpm per target cell. Target cells were washed three times with cold RPMI-FBS and resuspended at a concentration of 5×10^4 cells per ml in the same medium.

Uninfected, ^{51}Cr-labeled FS_4 cells prepared in a similar manner served as control target cells.

NK cytotoxicity assay. The chromium-51 release assay for NK activity was performed with HSV-1-infected and uninfected ^{51}Cr-labeled FS_4 target cells in flat-bottomed microtest plates (Costar 3596, Cambridge, Mass.). In each well, 5×10^3 target cells in 100 μl were planted immediately before adding 100 μl of effector cells at concentrations resulting in 100:1, 50:1, 25:1, 12:1, 6:1, and 3:1 effector to target cell ratios (E:T). Experiments with infected and uninfected target cells were performed in triplicate. Each test included the following: (i) spontaneous ^{51}Cr release from HSV-1-infected and uninfected target cells with medium alone (RPMI-FBS) in the absence of effector cells; (ii) total ^{51}Cr release from each of the two target cells by lysis with 100 μl of a 5% solution of Triton X (New England Nuclear Corp., Boston, Mass.) in RPMI-FBS.

All reaction mixtures were incubated 14 to 16 h at 37°C in a humidified 5% CO_2 atmosphere. After incubation, 100 μl of supernatant was collected from each well and the ^{51}Cr activity was determined by scintillation spectrometry (Beckman model LS 3133P) using Biofluor scintillation fluid (New England Nuclear Corp., Boston, Mass.).

The percent ^{51}Cr release was determined using the following formula. Percent ^{51}Cr release = (experimental release − spontaneous release)/(total release − spontaneous release) × 100.

Experimental release represents the actual number of counts per minute obtained from the incubation of target cells and lymphocytes in experimental wells; spontaneous release represents ^{51}Cr counts per minute released by target cells in the absence of effector cells; total release represents the total ^{51}Cr counts per minute released by target cells incubated with Triton X-100. The percent specific release (%SR) was calculated by subtracting the percent ^{51}Cr release with uninfected cells from the percent ^{51}Cr release with HSV-1-infected cells. The %SR was determined to specifically define ^{51}Cr release attributable to HSV-1 infection of the target cells. Only experiments demonstrating less than 35% spontaneous ^{51}Cr release (approximately 80% of the experiments) were used.

Experimental and control values were expressed as the average of at least three triplicates. Standard deviations of the triplicates usually fell into the range of 2 to 7% and almost never exceeded 10%. The data were analyzed by Student's *t* test on raw data.

Depletion of adherent cells. Mononuclear cells were depleted of adherent cells by the method of Ly and Mishell (20), as modified by Berlinger et al. (3). Briefly, mononuclear cells were suspended in RPMI with 20% FBS and antibiotics and were passaged through a column of Sephadex G-10 beads equilibrated in the same medium.

Depletion of B-cells. Peripheral blood mononuclear cells separated on a Ficoll-Hypaque gradient were passed through nylon-wool columns according to the method of Julius et al. (12). Cell suspensions containing 30×10^6 to 50×10^6 cells in 2 ml of RPMI-FBS were slowly loaded onto nylon-wool columns, layered with 1 ml of warm medium, and sealed with Parafilm before being incubated at 37°C for 45 min. The cells were eluted slowly with 25 ml of warm medium (RPMI-FBS), pelleted, washed twice, and enumerated.

Depletion and recovery of T-cells. The capacity of T-cells to spontaneously form rosettes with sheep erythrocytes was utilized for separating them from other lymphoid cells (11). One hundred microliters of a lymphocyte suspension (5×10^6 cells per ml) was

mixed with 100 μl of 1% sheep erythrocytes and 25 μl of heat-inactivated FBS (absorbed earlier with sheep erythrocytes). This mixture was incubated at 37°C for 5 min, centrifuged at 50 × *g* for 5 min, and then incubated at 4°C for 1 h. The pellet was gently resuspended and the rosetted cells were centrifuged on a second Ficoll-Hypaque gradient at 400 × *g* for 30 min to separate the rosetted cells from the non-rosette-forming cells. The pellet (T-rosette-forming cells) and the interface cells (non-T) were harvested separately. The erythrocytes in the pellet were lysed by hypotonic shock. These cells and the interface cells were washed twice, and the cell suspensions were adjusted to 5 × 10^6 cells per ml in RPMI-FBS.

Surface immunoglobulins as a marker for enumerating B-cells. Fluorescein isothiocyanate-labeled polyvalent antiglobulin serum (Hyland Laboratories, Inc., Costa Mesa, Calif. and Meloy Laboratories, Springfield, Va.) was utilized for enumerating B-cells (10). Macrophages, which bind antiglobulin by Fc receptors, were differentiated from B-cells by phagocytosis of latex beads (3).

RESULTS

Characteristics of natural kill for HSV-1-infected target cells. Experiments were carried out to determine the incubation time required for development of optimum NK(HSV-1) activity. Normal donor lymphocytes were incubated with HSV-1-infected or uninfected target cells at an E:T of 50:1 for various periods of time (Fig. 1A). ^{51}Cr release with infected and uninfected target cells was minimal after 4 h of incubation, increased sharply at 12 h of incubation, and plateaued after 18 h of culture; whereas ^{51}Cr release with the uninfected target cells increases steadily during the entire 24-h incubation period. The %SR was calculated (percent ^{51}Cr release with infected target cells minus ^{51}Cr release with uninfected target cells) for each time point (Fig. 1B) and was found to be maximal at 12 h of incubation and to decrease slowly thereafter. These observations indicated that the optimum time of incubation was about 12 to 14 h.

Figure 1B also demonstrates the progressive increase with time of the spontaneous release of ^{51}Cr from HSV-1-infected target cells. To keep the spontaneous ^{51}Cr release below 35%, the NK(HSV-1) assay had to be harvested before 18 h of culture.

Figure 2A demonstrates a typical NK(HSV-1) assay using lymphocytes from a seropositive and a seronegative individual. ^{51}Cr release with HSV-1-infected target cells was significantly higher than ^{51}Cr release with uninfected target cells at E:T of 25:1, 50:1, and 100:1 (for each, $P < 0.001$). The %SR for each individual is shown in Fig. 2B. Since the ^{51}Cr release at the 50:1 E:T was always on the straight portion of the graph, it

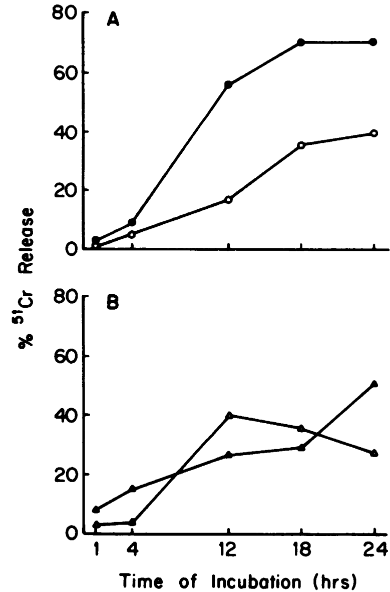

FIG. 1. *Time course of development of NK(HSV-1) in culture. (A) Mononuclear cells from normal seropositive control were incubated with HSV-1-infected (●) or uninfected (○) fibroblasts at an E:T of 50:1. The ^{51}Cr release assay was harvested after incubation for various periods of time. (B) The %SR (% SR [△] defined as percent ^{51}Cr release with HSV-1-infected target cells minus percent ^{51}Cr release with uninfected target cells) was calculated for each time period of incubation. Also included was the percent spontaneous ^{51}Cr release found after the various periods of incubation with the HSV-1-infected cells (▲).*

was used for comparing results of various individuals.

NK versus ADCC. Since most normal adults have antibody to HSV-1, cytophilic antibody or antibody produced in vitro might be present in sufficient concentrations to result in antibody-dependent kill of target cells. This possibility is supported by the very low levels of antibody required for ADCC (33). Since even low concentrations of antibody to HSV-1 might result in ADCC, it was important to determine whether the NK(HSV-1) assay was detecting any antibody-dependent lysis. The NK(HSV-1) of 22 seropositive individuals was compared to that of 8 seronegative controls (Fig. 3). Clearly, the seropositive individuals demonstrated an average NK(HSV-1) significantly higher than that of the seronegative controls ($P < 0.01$) when evaluated as percent ^{51}Cr release using HSV-1-infected target cells or as %SR.

Møller-Larsen et al. (21) showed that ADCC to HSV-1-infected skin fibroblasts could be reduced by extensive washing of the effector cells

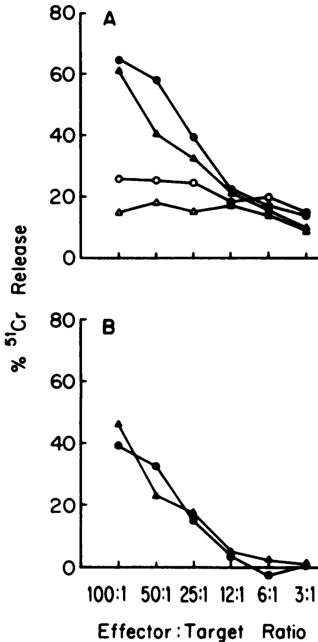

FIG. 2. *Results of a typical NK(HSV-1) assay utilizing isolated mononuclear cells. (A) (●, ▲), ^{51}Cr release with HSV-1-infected target cells. (○, △), ^{51}Cr release with uninfected target cells. In this experiment, a seropositive control (●, ○) was compared to a seronegative control (▲, △). ^{51}Cr release was significantly ($P < 0.001$) greater with HSV-1-infected versus uninfected targets at E:T cell ratios of 25:1, 50:1, and 100:1. (B) The %SR was calculated for the seropositive (●) and the seronegative (▲) controls. A similar result was obtained using linear regression of points (9% difference of %SR at 50:1 [E:T]).*

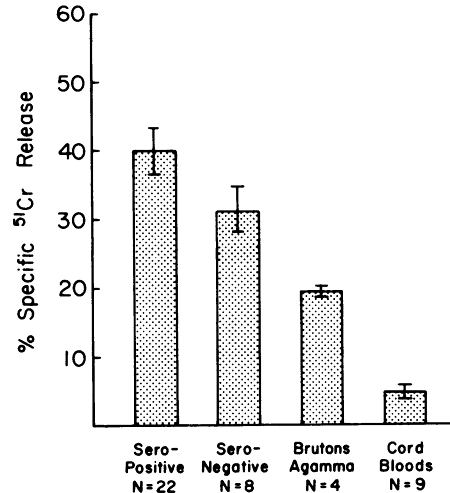

FIG. 3. *Comparison of the average %SR obtained by the study of seropositive and seronegative controls, patients with Bruton's agammaglobulinemia, and unresponsive cord blood samples. Error terms ± 1 standard error. The ^{51}Cr release with infected and uninfected cells was: 53 and 13% for seropositive controls, 44 and 13% for seronegative controls, 28 and 8% for agammaglobulinemia patients, and 14 and 10% for unresponsive cord blood samples.*

from seropositive individuals. Experiments were therefore carried out to determine whether washing effector cells seven times rather than twice diminished NK(HSV-1) for seropositive individuals. The NK(HSV-1) of the seropositive individual was not changed, whereas similar treatment of effector cells from a seronegative individual resulted in a slightly increased %SR caused mostly by a small decrease in ^{51}Cr release found with the uninfected target cells (data not shown).

Since antibody specific for HSV-1 might be produced during the long culture period and could also result in ADCC, experiments were carried out to determine whether depletion of B-cells resulted in decreased NK(HSV-1) activity for effector cells from seropositive, but not seronegative, individuals. Passage of leukocytes over a nylon-wool column reduced by 80 to 90% the number of immunoglobulin-bearing cells (B-cells). Treatment of effector cells from seropositive individuals consistently resulted in a decrease of both percent ^{51}Cr release with infected target cells and in a decrease of %SR. Conversely, passage over nylon of effector cells from seronegative controls consistently resulted in an increase in both ^{51}Cr release with infected targets and %SR (Table 1). Table 1 demonstrates the results of one typical experiment; four similar experiments have been carried out with comparable results.

Four patients with Bruton's agammaglobulinemia were also evaluated and found to have NK(HSV-1) responses significantly below those of seronegative ($P < 0.01$) or seropositive ($P < 0.001$) individuals (as evaluated by ^{51}Cr release with HSV-1-infected cells or as %SR). By comparison, mononuclear cells prepared from 9 of 12 cord blood samples demonstrated NK(HSV-1) responses significantly lower ($P < 0.001$) than the normal controls or agammaglobulinemia patients (Fig. 3). The average %SR for the 9 unresponsive cord blood samples was 4% (range, 0 to 12%). Only 3 of the 12 cord blood samples tested demonstrated a response which fell within the normal range (average response of seronegative controls ± 2 standard deviations, 14 to 47%; or average response of agammaglobulinemia patients ± 2 standard deviations, 15 to 23%).

Characteristics of the NK(HSV-1) cells. Passage of effector cells over nylon-wool columns effectively reduced the percentage of B-

TABLE 1. *Effect on NK(HSV-1) activity of passage of effector cells over nylon-wool columns*

Lymphocyte donor	^{51}Cr release (%)					
	Unfractionated			Nylon-wool eluted[b]		
	HSV-1 infected	Uninfected	Specific release	HSV-1 infected	Uninfected	Specific release
Seropositive						
no. 1	52 ± 3.9[a]	10 ± 5	42	42 ± 3.3[c]	6 ± 1.2[c]	36
no. 2	45 ± 3.8	8 ± 2	37	37 ± 5.3[c]	12 ± 2.8[c]	25
Seronegative						
no. 1	33 ± 5.8	9 ± 2.8	24	40 ± 5.1[c]	6 ± 1.5[c]	34
no. 2	50 ± 5.0	20 ± 3.2	30	62 ± 5.4[d]	17 ± 2.8[c]	45

[a] ± Standard error of the mean.
[b] Nylon-wool–eluted cells were shown to be depleted, by 80 to 90%, of immunoglobulin-bearing cells (B-cells) and monocytes.
[c] $P > 0.1$; change due to nylon-wool filtration was not significant.
[d] $P < 0.05$; change due to nylon-wool filtration was significant.

cells (immunoglobulin-bearing cells) and adherent cells without abolishing NK(HSV-1) activity (Table 1). These results indicated that the effector cells were neither B-cells nor macrophages. In addition, passage of effector cells over Sephadex G-10 beads, a procedure which removed more than 90% of the latex (0.801 μm)-ingesting cells, also failed to remove the active cells (data not presented), further indicating that macrophages were not the effector cells.

Experiments were also carried out to determine whether the effector cells for NK(HSV-1) expressed the surface markers of T-cells. Effector cells from seronegative and seropositive individuals were passed over nylon-wool and then depleted of cells which spontaneously rosetted with sheep erythrocytes. The cells which rosetted (T-cells) and the nonrosetting cells (non-T-cells) were assayed for NK(HSV-1) activity. Amost all of the activity was associated with the non-T-cell fraction (Table 2).

TABLE 2. *Capacity of T-cells and non-T-cells to act as effector cells in the NK(HSV-1) assay*

Lymphocyte donor	^{51}Cr release[a] (%)		
	HSV-1 infected	Uninfected	Specific release
Seropositive			
Unfractionated	35 ± 6.5[b]	3 ± 1.6	32
Nylon eluted	25 ± 4.3	3 ± 2.5	22
T-cells	20 ± 4.4	3 ± 1.4	17
non-T-cells	45 ± 3.7	4 ± 1.7	41
Seronegative			
Unfractionated	35 ± 3.8	10 ± 3.5	25
Nylon eluted	40 ± 3.7	12 ± 2.6	28
T-cells	4 ± 4.4	2 ± 3.6	2
non-T-cells	63 ± 4.1	1 ± 2.5	52

[a] E:T of 12:1.
[b] ± Standard error of mean.

Low NK(HSV-1) and severe herpesvirus infections. Our findings that lymphocytes from most (9 of 12) cord blood samples demonstrated low or absent NK(HSV-1) is of particular importance since this may be associated with the well-known susceptibility of newborn infants to severe and life-threatening infections with the herpesviruses (21). To determine whether this observation could be extrapolated to adults susceptible to severe herpesvirus infections, studies were carried out with two patients with no known primary or secondary immunodeficiency disease but with severe, disseminated HSV-1 infections. At the time of these severe infections, lymphocytes from these young adults demonstrated an NK(HSV-1) response of 10 and 11% (%SR) which was greater than 2 standard deviations below the mean for normal seropositive or seronegative individuals and were similar to the responses of several of the unresponsive cord blood samples tested. (The conclusion was the same whether the %SR or percent ^{51}Cr release with HSV-1-infected cells was used in the evaluation.) By comparison, we found that normal individuals suffering from virus infections (either reactivated herpes labialis or upper respiratory tract infections) demonstrated elevated ^{51}Cr release with HSV-1-infected target cells and usually elevated %SR (Lopez et al., manuscript in preparation). The latter observations are analogous to those of Welch (37) in virus-infected mice.

DISCUSSION

NK cells are a population of lymphoid cells which appear to play an important role in resistance to syngeneic tumors in the mouse (7).

As described in mice and humans this cell population lacks the surface markers and other properties usually associated with T-cells, B-cells, or macrophages (9, 25, 37). Studies with mice indicate that the NK cell is bone marrow-dependent and can be abolished by treatment of the mice with ^{89}Sr (8).

Resistance to HSV-1 in the mouse is also bone marrow-dependent and has been shown to be abrogated by treatment of the mice with ^{89}Sr (16). An NK assay using HSV-1-infected target cells was developed to determine whether such an assay might reflect resistance to HSV-1 in mice. Preliminary studies with the mouse model indicated that the NK (HSV-1) assay reflected resistance (16) and that fibroblasts of either H-2b or H-2d mice worked equally well in the assay. Since there appeared to be no genetic restriction, we felt that a similar assay could be developed with human fibroblasts and used to determine whether the results of such an assay might reflect the individual's capacity to resist HSV-1 infections.

In developing the NK(HSV-1) assay, our greatest concern was that this assay might be evaluating ADCC rather than natural kill. Clearly, others have developed sensitive ADCC assays for both antibody titers to HSV-1 and for killer (K)-cell function (26, 31). It is equally clear that the ADCC capacity of individuals does not reflect their capacity to resist HSV-1 infections. Thus, Russell and Essery (28) showed that patients with malignant reticuloendothelial diseases had normal K-cell function. Shore et al. (32) have also shown normal K-cell function in cord blood lymphocytes which, again, does not reflect the susceptibility of the newborn to HSV-1 infections. Because of these observations, any portion of our results due to ADCC had to be clearly identified, and procedures had to be developed to remove this activity from the assay.

On the other hand, our studies have shown that antiviral antibody is not required for NK(HSV-1) activity. Thus, individuals with no detectable antibody to HSV-1 and without the capacity to respond to HSV-1 antigen in the blast transformation test (17) were capable of normal NK(HSV-1) responses. Additionally, patients with Bruton's agammaglobulinemia, some with serum immunoglobulin G concentrations as low as 25 mg/100 ml, also demonstrated good NK(HSV-1) responses. We have also recently tested a patient with severe combined immunodeficiency (without detectable T- or B-cells). This patient demonstrated normal NK(HSV-1) (%SR = 27) despite the lack of B- and T-cells (Lopez et al., submitted for publication).

Although patients with Bruton's agammaglobulinemia demonstrated significant NK-(HSV-1) function, it is clear that the percent ^{51}Cr release with infected target cells and %SR were significantly lower than the values for normal seronegative controls. It is difficult to know whether this result is caused by a suppressor cell or whether there is a deficiency of NK(HSV-1) somehow associated with the primary immunodeficiency. Further studies must be carried out with these patients to better understand these results.

In many ways our study confirms the published reports of Santoli and co-workers (29, 30). We must disagree, however, with their conclusion that antibody to the virus probably does not play a role in this test (30). Our studies showed that seropositive individuals responded with significantly higher NK(HSV-1) than seronegative individuals (Fig. 3) and that removal of B-cells from the former reduced the response, whereas removal of B-cells from the seronegative individuals increased their response. Although other interpretations are possible, a small amount of antiviral antibody is probably being made by the B-cells resulting in antibody-dependent kill. These results indicate that B-cell removal will probably be required in studies comparing NK(HSV-1) of seropositive individuals.

The target(s) for the lysis of tumor cells by NK cells are under study in several laboratories. The target structures (antigen?) on the YAC tumor cells appear to be three nonviral, cell-surface glycoproteins (27). About 50 new proteins are expressed in HSV-1-infected cells (23), and only about 28 of these are found incorporated into virions (13). The specific target for the NK(HSV-1) function may, therefore, be any of the structural or nonstructural proteins in the infected cell. The strain distribution of mice capable of a strong NK(YAC) response (13) is different from the strain distribution of mice resistant to HSV-1 (14). If the NK(HSV-1) assay is found to reflect resistance to HSV-1 in the mouse and if nonstructural antigens are the targets of NK lysis in HSV-1-infected target cells as was found in YAC tumor cells, then the specific target(s) expressed by the latter may differ from those expressed on HSV-1-infected cells and thus may define the differences found in strain distribution. In other words, NK(HSV-1) may depend on a target not shared by YAC tumor cells or the latter may have targets not shared by HSV-1-infected cells. Strains of mice having NK cells unable to recognize one target or the other would be unable to respond to that target and would thus be susceptible to that tumor cell or virus.

Our finding of low NK(HSV-1) responses in patients with severe herpesvirus infections are preliminary but parallel our studies showing low NK(HSV-1) in mice susceptible to HSV-1 (16). Although these results could be interpreted to mean that the virus infection suppressed the natural kill, our studies demonstrating high NK(HSV-1) in normal individuals with virus infections dispute this possibility. The possibility thus exists that low natural kill in the two individuals before infection was causally related to their susceptibility to the severe herpesvirus infections. The small number of patients studied precludes firm conclusions, and further studies are required to establish in a definitive way whether low NK(HSV-1) is associated with susceptibility to herpesvirus infections.

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