INCREASED SENSITIVITY OF HUMAN LYMPHOID LINES TO NATURAL KILLER CELLS AFTER INDUCTION OF THE EPSTEIN-BARR VIRAL CYCLE BY SUPERINFECTION OR SODIUM BUTYRATE*

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A subfraction of human blood lymphocytes can lyse certain established cell lines in tissue culture in short-term assays (1-4). The system is similar to the natural killer (NK) cell system in rats and mice (5, 6). Unlike cytotoxic T cells directed against virally determined antigens or haptens, NK cells do not appear to be under any major histocompatibility complex restriction (7). The NK effect is exerted by a subset within the T-cell lineage (8-10). Most, but not all of them, carry Fc receptors (8, 11, 12) and some carry C3 receptors as well (12, 13).

The panel of highly NK-sensitive cell lines in men and mice shows a somewhat erratic pattern. T cell-derived human lymphoid lines are often more sensitive than B cell-derived lines. NK activity in mice was found to increase after virus infection, treatment of the effector cells with interferon or interferon inducers, and tumor-cell inoculation (14-16). Several recent reports suggest that viral infection of the target cells increases their NK sensitivity (17, 18). Highly NK-active nude mice were found to reject persistently virus-infected tumor cells, in contrast to their noninfected counterparts (19), this rejection was attributed to NK cells.

It is thus conceivable, and even likely, that NK cells play a role in restricting viral infections by attacking virally infected cells and nipping them in the bud, as it were. Although the evidence for such an effect is highly suggestive, it is far from conclusive.

This paper addresses itself to the question of whether NK cells may play a role in restricting the spread of a virus that has prolonged latency as a major part of its natural life cycle, with only occasional entry of the virus-carrying cells into the productive cycle. If NK cells play any restrictive role in limiting virus spread, it could be expected that they would kill cells that have already entered the cycle more efficiently than cells that carry the viral genome in a latent state.

Epstein-Barr virus (EBV), a lymphotropic herpesvirus in man, appears to be particularly well suited for the study of this question. In primary infection, as it occurs in infectious mononucleosis, it infects B lymphocytes and converts them into Epstein-
Barr-virus-determined nuclear antigen (EBNA) positive cells. The patients respond with antibody production against viral capsid antigen (VCA), early antigen (EA) and, eventually, against EBNA in that temporal order. Later, as the acute infection subsides, the number of virus-carrying cells falls (20) and stays at a low level in a latent form over a patient's lifetime. After the acute disease, antibodies against VCA and EBNA persist, whereas the anti-EA response fades away.

The mechanisms that keep the number of virus-producing cells at a relatively low level are not known. If NK cells play a role, one would expect that EBV-DNA-carrying cells that have entered the viral cycle would be more sensitive to NK lysis than the progenitor cells that carry the viral genome in a latent form.

EBV-carrying lymphoblastoid and Burkitt's lymphoma-derived lines all contain the viral genome in multiple copies (21-23), and express EBNA (24). However, only a very small proportion, if any, of the cells enter the viral cycle at a given time. This proportion can be dramatically increased, however, by superinfecting EBV-carrying cultures with massive doses of the P3HR-1 substrain of EBV (25), or by treatment with certain chemical inducers, such as n-butyrate (26-30).

In this paper, we show that both methods of EBV-cycle induction lead to an increase in the NK sensitivity of EBV-carrying cell lines. Interestingly, superinfection with a transforming virus prototype, B95-8, which does not increase the fraction of cells entering the viral cycle, had no such effect (with one exception).

Materials and Methods

Virus. Infectious EBV from the P3HR-1 cell line was provided by the Pfizer Chemical Div., Pfizer, Inc., New York, under a contract from the Division of Cancer Cause and Prevention, National Cancer Institute, National Institutes of Health, Bethesda, Md.

The virus was received in wet pack, frozen, and stored at -70°C. These preparations usually induced 20-30% EA-positive cells in the Raji line 48 h after superinfection with a 1:75 dilution. B95-8 virus was concentrated by centrifugation at 9,000 rpm for 2 h from the supernate of B95-8 cells; it was frozen and stored at -70°C. Its infectivity was assayed by exposing the EBV-negative Ramos cell line to 1:5 and 1:10 dilutions; 24-27% EBNA-positive cells were induced after 48 h (31).

Cell Lines. All cells were propagated as suspension cultures in RPMI-1640 medium with 8% fetal calf serum (Grand Island Biological Co., Grand Island, N. Y.) and subcultured twice weekly. Raji (32) and Daudi (33) originated from Burkitt's lymphoma, BJAB (34) was derived from an EBV-negative Burkitt's lymphoma, Molt-4 (35) was derived from an acute lymphocytic leukemia patient, K562 (36) was established from a patient with chronic myeloid leukemia. Both AT-A-CL7-III and NAD-7 were established in our laboratory, by transforming the blood lymphocytes of an ataxia telangiectasia patient and a healthy adult. Both lines were transformed with the B95-8 strain of EBV.

Superinfection of Cells. Cells were suspended at a concentration of 1 x 10⁶ per ml and infected with the appropriately diluted virus for 1 h at 37°C. The cells were resuspended in culture medium at a concentration of 0.5 x 10⁶ per ml and incubated at 37°C for 24 h. Cells superinfected with P3HR-1 virus are designated by adding the symbol (V).

Demonstration of EBV-associated Antigens. EA was stained on acetone-fixed smears with an fluorescein isothiocyanate-conjugated IgG fraction of EA- and VCA-positive human serum at a dilution of 1:40 as previously described (37). EBNA was stained by anti-complement immunofluorescence on methanol-acetone-fixed smears (24).

Lymphocytes. Lymphocytes were separated from heparinized blood by the Ficoll (Pharmacia Fine Chemicals, Div. of Pharmacia, Inc., Piscataway, N. J.) -Isopaque (Nyeggard, & Co. A/S, Oslo, Norway) method of B6yum (38).

Cytotoxic Assay. Chromium release tests were performed in a Falcon II microplate (Falcon Labware, Div. Becton, Dickinson & Co., Oxnard, Calif.) (39). Peripheral blood lymphocytes
(PBL) were used as effector cells at a 100:1 effector target ratio. \(5 \times 10^3\) target cells were added to each well in a vol of 0.1 ml followed by the addition of \(5 \times 10^5\) PBL per 0.1 ml. Finally, 0.1 ml medium was added to each well to give a final concentration of 0.3 ml per well. Test combinations were set up in quadruplicate. Control wells containing only target cells were included in each experiment to determine maximum and spontaneous release. The microplate was incubated at 37°C in a 5% CO2 atmosphere for 4 h. After incubation, the plate was centrifuged at 1,500 rpm for 5 min. 0.1 ml of the cell-free supernate was removed. The specific \(^{51}\text{Cr}\) release was determined according to the following formula:

\[
\frac{\text{release in test} - \text{spontaneous release}}{\text{maximal release} - \text{spontaneous release}} \times 100.
\]

Experiments in which the spontaneous release was >25% were not evaluated.

**Cold-Target Inhibition of Cytotoxicity.** The cytotoxic test was performed as above after graded numbers of unlabeled cells were added simultaneously with the target cells in 0.1 ml medium. The microplate was rocked on a Bello (Bello Glass, Inc., Vineland, N. J.) rocker at a slow speed during the duration of the assay. The results were expressed as the percentage of inhibition.

**Induction of Cells by n-Butyrate.** 3 mM n-butyrate was added to P3HR-1 cells suspended at a concentration of 0.4 \(\times 10^5\) per ml for various time intervals.

**Virus Neutralization.** P3HR-1 virus was incubated with 1:10 anti-EBV (VCA) -positive or -negative sera at 4°C for 2.5 h. The final concentration of the virus was the same as in the superinfection experiments. After incubation, the infection experiment was performed as usual.

**Kinetics.** Aliquots of Raji cells from one pool were removed and infected at different time intervals with the P3HR-1 virus derived from the same batch. All cells were harvested simultaneously, \(^{51}\text{Cr}\) labeled, and exposed to the same effector lymphocytes.

## Results

**P3HR-1 Virus Superinfection Increases the NK Sensitivity of Raji Cells.** Raji cells are relatively resistant to NK killing (40). EBV (P3HR-1 strain) superinfection increases the NK sensitivity of Raji cells after 24 h (Fig. 1). This was observed in all 25 experiments that were carried out. The mean percent specific chromium release increased from 3.4 to 27%. There was no increase of the background \(^{51}\text{Cr}\) release. The addition of human serum instead of fetal calf serum did not influence the results. Raji cells superinfected with different concentrations of virus showed increased specific \(^{51}\text{Cr}\) release depending on the virus concentration (Fig. 2).

**The Virus-induced Increase in NK Sensitivity Depends on the Efficiency of Infection.** P3HR-1 virus superinfection induced the entry of a certain proportion of Raji cells into the viral cycle, as indicated by the appearance of EA. To examine if there was any relationship between the efficiency of the superinfection, as measured by the EA-induction criterion, and the increased NK sensitivity, aliquots of the culture tested for NK sensitivity were fixed 48 h after infection and the frequency of EA-positive cells was determined. The relationship between the frequency of EA-positive cells and NK sensitivity is shown in Fig. 3. The results suggest that the sensitivity of the target cells depends on the initiation of the viral cycle (although not necessarily on EA synthesis as such).

**Virus Neutralization Test.** Because superinfection was carried out with concentrated, virus-containing P3HR-1 culture supernate and not with purified virus, it had to be checked whether the virus itself could be held responsible for the increased NK-sensitivity. Infection tests were therefore also performed with preparations exposed to EBV antibody-positive human serum, for 2½ h before infection (Table I). EBV antibody-positive, but not negative, serum abolished the ability of the virus-containing
FIG. 1. Cytotoxic effect of human blood lymphocytes on Raji (V) cells. The cells superinfected in fetal calf serum (FCS)- and in human serum (HS)-containing medium are shown for comparison. Each point represents a different experiment, for each target cell. Means and standard errors are shown. The cytotoxicity was measured as percent specific chromium release at a 100:1 effector:target cell ratio in a 4-h assay, 24 h after infection. The mean percent spontaneous release was 18.2 ± 3.6 for uninfected and 18.5 ± 3.6 for Raji (V) cells, in 25 experiments.

Supernates to increase the NK sensitivity, indicating that the virus was responsible for the effect.

Changes in NK Sensitivity in Raji Cells as a Function of Time After Superinfection. The NK sensitivity of P3HR-1-virus-superinfected Raji cells increased by 12 h, peaked at 24 h, and declined subsequently (Fig. 4). Because viral attachment to the EBV surface receptors is maximal immediately after the infection (41) and declines thereafter, the sensitivity is not merely a result of the presence of attached virus particles on the cell surface.

Superinfection of Other Cell Lines. Four other lymphoid lines were also superinfected with P3HR-1 virus. EA induction served as the measure of successful infection. The different cell lines varied both with regard to the induction of EA and of NK sensitivity. Experiments with the lymphocytes of two donors differing in NK efficiency are given as examples in Table II.

Comparison between Effector Cells of EBV Seropositive and Seronegative Donors. It can be
assumed that the increased NK sensitivity of the EBV-superinfected Raji cells is a result of the recognition of EBV-associated antigens by immune lymphocytes, or of an antibody-dependent cell-mediated (ADCC) mechanism. In the latter case, antibodies would be secreted during incubation. Therefore the cytotoxicity of effector cells derived from EBV-seropositive and seronegative donors was compared in four experiments (Table III). There was no significant difference between the killing effect of cells from EBV-positive and -negative donors. Thus, although EBV is responsible for the induction of NK sensitivity (as indicated by the virus neutralization test), cytotoxicity is not a result of a preimmunized state of the lymphocyte donor against EBV.

**Cold-Target Inhibition Tests.** The question arose whether the killing of the EBV-superinfected Raji was a result of the effector-target interaction operating also in the
known human NK system. As one approach toward this question, we have performed cold target inhibition tests with two cell lines known for their high NK-sensitivity, Molt-4 and K562. The cell populations used for competition were tested for their NK sensitivity in parallel. We could readily confirm the high NK sensitivity of these lines (specific $^{51}$Cr release: 35-40% at a 100:1 effector:target cell ratio). BJAB had low NK sensitivity (6-9%), Daudi and Raji were even lower (1-3%), and NAD-7 was insensitive (0-1%). In the competition test, cytotoxicity against Raji (V) was inhibited by the homologous cell, Raji (V) itself, and by Molt-4 and K562 (Fig. 5). BJAB inhibited the cytotoxicity to a lesser extent; NAD-7 and Daudi did not inhibit at all. It is of interest that the uninfected Raji showed a slight but significant inhibition. Thus with the exception of Raji, the capacity of the cell lines to inhibit the killing of Raji (V) was directly related to their NK susceptibility as targets.

We have also performed the reciprocal experiment with Molt-4 as targets. Cell-mediated lysis of the Molt-4 could be inhibited by both Raji (V) and Daudi (V), whereas uninfected Raji and Daudi were ineffective (Fig. 6). In keeping with their high NK sensitivity, Molt-4 and K562 were more efficient competitors than Raji (V). It is noteworthy that uninfected Raji cells did not compete in this system although they did compete against superinfected Raji cells as already mentioned (compare Fig.

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**Table I**

_Virus Neutralization Before Superinfection Prevents Increased NK Sensitivity_

<table>
<thead>
<tr>
<th>Cells</th>
<th>Virus treatment</th>
<th>Percentage of EA</th>
<th>Percentage of $^{51}$Cr release</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raji control</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raji (V)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raji</td>
<td>Infected with virus exposed to EBV antibody-negative serum</td>
<td>29</td>
<td>25</td>
</tr>
<tr>
<td>Raji</td>
<td>Infected with virus exposed to EBV antibody-positive serum</td>
<td>12</td>
<td>5</td>
</tr>
</tbody>
</table>

P3HR-1 virus was incubated with EBV antibody-positive serum (VCA titer: 160, EA: < 10, EBNA: 80) and EBV antibody-negative serum (VCA titer: < 10, EA: < 10, EBNA: < 2) for 2.5 h before infection.

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**Fig. 4.** Time-course of the acquisition of sensitivity to the lymphocyte killing. Raji cells taken from one constant pool were infected at varying time intervals by aliquots from one P3HR-1 virus preparation. These populations Raji (V) (O) and Raji (O) were simultaneously harvested and assayed with the same effector lymphocytes.
EPSTEIN-BARR VIRUS SUPERINFECTION INCREASES SENSITIVITY

### Table II
NK Sensitivity of Different Cell Lines After Infection with P3HR-1 Virus

<table>
<thead>
<tr>
<th>Line</th>
<th>Percentage of EA positive cells, experiment No</th>
<th>Percentage of specific $^{31}$Cr release, experiment No</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Raji (V)</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Raji</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Daudi (V)</td>
<td>41</td>
<td>45</td>
</tr>
<tr>
<td>Daudi</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>BJAB (V)</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>BJAB</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>AT-A-CL7-III (V)</td>
<td>11</td>
<td>15</td>
</tr>
<tr>
<td>AT-A-CL7-III</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>NAD-7 (V)</td>
<td>21</td>
<td>15</td>
</tr>
<tr>
<td>NAD-7</td>
<td>0</td>
<td>5</td>
</tr>
</tbody>
</table>

100:1 effector:target cell ratio in a 4-h assay 24 h after superinfection.

### Table III
Comparison of the Cytotoxicity of Lymphocytes Obtained from EBV Antibody-positive and EBV Antibody-negative Donors

<table>
<thead>
<tr>
<th>Lymphocyte donor*</th>
<th>Experiment No.</th>
<th>EBV antibody-positive serum</th>
<th>EBV antibody-negative serum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Raji</td>
<td>Raji (V)</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>15</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>24</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>37</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>0</td>
<td>17</td>
</tr>
<tr>
<td>Mean</td>
<td>0.5</td>
<td>21.3</td>
<td>1.0</td>
</tr>
</tbody>
</table>

* Percentage of specific $^{31}$Cr release of Raji and Raji (V) targets. The lymphocytes of seven EBV antibody-positive and 4 EBV antibody-negative donors were tested.

5). The cold-target inhibition experiments indicate that the cytotoxicity against the superinfected cells is mediated by the same cells as the NK phenomenon.

Superinfection with Transforming (B95-8) Virus. The P3HR-1 virus is unique among EBV isolates in its ability to induce an abortively lytic cycle in Raji and certain other cell lines. Wild type virus transforms normal B lymphocytes into established lines, induces EBNA, but does not cause the entry of EBV-carrying cell lines into the cycle,
nor does it induce any detectable EA. We tested the effect of transforming-B95-8 viral superinfection on the NK sensitivity of Raji cells. B95-8 virus was titrated for infectivity by its ability to induce EBNA in the EBV-negative Ramos line (31). Two different virus pools were tested, one of them at three different concentrations. B95-8 virus exposure did not increase the NK sensitivity of Raji cells (Table IV). Daudi cells showed a certain increase in NK sensitivity although this was less dramatic than with P3HR-1 virus (compare Table II). EA values in the B95-8-infected Daudi cells, however, were not increased from the uninfected control cells (1%).

Virus Induction with n-Butyrate. Some EBV-carrying cells can be induced to express EA by iododeoxyuridine, bromodeoxyuridine, 12-O-tetradecanoyl-phorbol-13-acetate (TPA), cycloheximide, and n-butyrate (26-30). P3HR-1 cells could be induced more efficiently than Raji by n-butyrate as indicated by the appearance of EA (26). We have therefore selected P3HR-1 cell to test the effect of n-butyrate treatment on cytotoxic sensitivity. The experiment was complicated by the fact that P3HR-1 cells were sensitive to NK lysis. Their sensitivity was highly variable. This variation may be related to the variable proportion of the cells that enter spontaneously the lytic cycle. In spite of this variation, the experiments showed that n-butyrate induction
EPSTEIN-BARR VIRUS SUPERINFECTION INCREASES SENSITIVITY

**Table IV**

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Percentage of specific 51Cr release</th>
<th>Percentage of EA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Raji (B95-8)</td>
<td>Daudi (B95-8)</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>3</td>
</tr>
</tbody>
</table>

Mean: 0.6, 2.5, 1.0, 16.0

The virus was tested at three different concentrations. The virus used in experiments 1 and 2 induced 24% EBNA in Ramos cells at a 1:5 dilution. The virus used in experiments 3, 4, and 5 induced 27% EBNA in Ramos cells at a 1:10 dilution.

**Table V**

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Duration of n-butyrate treatment</th>
<th>Percentage of specific 51Cr release, control</th>
<th>Percentage of specific 51Cr release in n-butyrate-treated cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>h</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>1</td>
<td>24</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>24</td>
<td>5</td>
<td>11</td>
</tr>
<tr>
<td>3</td>
<td>24</td>
<td>46</td>
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<tr>
<td>4</td>
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<td>15</td>
<td>22</td>
</tr>
<tr>
<td>5</td>
<td>48</td>
<td>24</td>
<td>31</td>
</tr>
</tbody>
</table>

$4 \times 10^5$ P3HR-1 cells per ml were incubated with 3 mM n-butyrate for different time intervals. Sensitivity to killing by blood lymphocytes was measured as specific 51Cr release at a 100:1 effector:target cell ratio in a 4-h assay.

![Graph](image)

**Fig. 7.** Sensitivity of n-butyrate-treated cells. $0.4 \times 10^6$ P3HR-1 cells were induced with 3 mM n-butyrate and used as targets in a 4-h 51Cr-release assay at a 100:1 effector:target cell ratio. Induced cells are (○), control P3HR-1 cells are (□).
Induction with n-Butyrate Causes a Parallel Increase in NK Sensitivity and EA Expression

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Percentage of specific $^{51}$Cr release</th>
<th>Percentage of EA</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Induction for 24 h</td>
<td>5</td>
<td>15</td>
</tr>
<tr>
<td>Induction for 48 h</td>
<td>8</td>
<td>24</td>
</tr>
</tbody>
</table>

regularly increased cytotoxicity (Table V), as assessed after 24-48 h. When followed as a function of time during a 2-d period, sensitivity increased with time (Fig. 7), in parallel with the EA values (Table VI).

Thus, induction of the EBV cycle by both chemical and viral agents increases the NK sensitivity of the human lymphoid lines.

Discussion

NK cells in the blood or spleen lymphocyte population of healthy individuals or unmanipulated experimental animals affect, in vitro, certain tumor-derived cell lines. There is considerable interest in the NK effect because it may contribute to immune surveillance. In part, this assumption is based on the fact that highly sensitive cell lines are derived from tumors. High sensitivity is the property of cultured cells. This may indicate that NK-sensitive cells are eliminated in vivo and survive only in vitro, when released from the pressure of the NK cells.

The nature of the killing is unknown. The sensitive cell panel does not provide any clue for the cell surface structure that serves as the target. The majority of investigators agree that the NK system lacks selectivity. Lymphocytes of different individuals differ in their killing efficiency but the ranking order of relative sensitivity remains similar when a spectrum of targets are confronted with different effector cells.

Cell lines can be divided into three categories: (a) sensitive in short-term assay; (b) sensitive only in long-term assay; (c) insensitive. The difference between the first and second categories is probably quantitative, in that a target displays sensitivity to effectors with a certain threshold level of activity. During prolonged interaction, the potentially cytotoxic lymphocytes are activated to kill with a higher efficiency.

We have shown that the relatively low NK sensitivity of human EBV-carrying B-cell lines can be increased by superinfection with the abortively lytic P3HR-1 virus or with n-butyrate, but not with the transforming EBV-prototype, the B95-8 virus. The increased NK sensitivity was maintained in the presence of Ara-C (Unpublished results.). When considered together with the kinetics of the effect, the findings suggest that the increased vulnerability by NK cells may develop in relation to the entry of the cells into the viral cycle. If this mechanism also operates in vivo, it may obviously help in restricting the spread of virus, by killing potential virus-producing cells before they reach the stage where they can release mature virus particles.

Lymphocytotoxicity was truly natural, i.e., it did not depend on previous immunization with the virus or with viral antigens. Lymphocytes of EBV-seropositive and -seronegative donors were equally effective.
The relationship between the lysis of the virally superinfected cells and the NK system was most clearly demonstrated by cold-target competition experiments. They indicated that the same effector cells operated in both. The ability of other cell lines, including EBV-genome-negative lines, to compete for the cytotoxic effectors was clearly related to their NK sensitivity.

The mechanism of increased NK sensitivity in the virally infected cells is not known. It is not merely a sign of increased membrane fragility, because the spontaneous release did not differ between infected and noninfected target cells. Moreover, the cells were harvested 24-48 h after infection, at the time when there is not yet any appreciable cell death as a result of the superinfection process. We have excluded all cultures with <85% viable cells.

The most convincing demonstration of a specific membrane change after superinfection, as opposed to a general increase in membrane fragility comes from the competition test. Superinfected cells competed in a known NK system, the lysis of the high NK-sensitive target, Molt-4.

Although the mechanism by which EBV-superinfection increases NK sensitivity to the target is not known, extrapolation from experimental evidence indicates that interferon may play an important role (42). Brief interferon treatment of lymphocytes increases their lytic potential, so that they may even affect otherwise NK-insensitive targets (43). The possibility that interferon, produced by the virally infected cells, is responsible for the induction of the cytotoxic effectors is unlikely, because B95-8 virus, but not P3HR-1 virus, superinfection was found to act as an interferon inducer in EBV-carrying, latently infected lines (44). In fact, interferon was not detected in the supernates of the P3HR-1 virus superinfected Raji cells. The ability of the superinfected cells to compete in the system with Molt-4, as target, speaks for an induced membrane change that renders the cells more sensitive to NK lysis.

Recently Pearson et al. (39) showed Raji (V) cells are sensitive to antibody-dependent lymphocytotoxicity exerted by anti-EBV antibody-positive serum and lymphocytes of healthy donors. In current experiments, we could confirm these findings and found that the ADCC effect was superimposed, over and above the virally induced NK sensitivity.

Summary

Superinfection of latently Epstein-Barr virus (EBV)-carrying Raji cells with the P3HR-1 substrain of EBV, known to induce the entry of a substantial fraction of the cells into an abortively lytic cycle, increased the susceptibility of the cells to the natural killer (NK) effect of human blood lymphocytes. Reciprocal cold-target competition tests with known NK-cell sensitive and -resistant lymphoid cell lines showed that the increased susceptibility is a result of the appearance of an NK-sensitive target, rather than to a general increase in membrane fragility.

Lymphocytes of EBV-seropositive and -negative donors were equally effective killers against P3HR-1 virus-superinfected targets. EBV-induced NK sensitivity increased with time. It was a result of some event associated with the intracellular viral cycle, and not to the adherence of viral particles to the cell surface. Induction of EBV-carrying P3HR-1 cells to entry into the viral cycle with n-butyrate also increased

2 We are grateful to Dr. Wolfgang Berthold of the Karolinska Institute for testing the supernates of Raji (V) for the presence of interferon.
their NK sensitivity. A transforming, noncytopathic prototype strain of EBV, B95-8, failed to increase the susceptibility of the Raji cells to NK-lysis, although it had some effect on the Daudi line.

Because NK cells can kill virus-producing cells at an early stage of the cycle, before the virus particles are assembled, they may restrict, in vivo, the spread of the virus from latently infected cells.

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