Natural killer cell-mediated lysis of T cell lines chronically infected with HIV-1

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SUMMARY

The susceptibility of HIV-1-infected $CD4^+$ T cell lines to natural killer (NK) cell-mediated lysis was examined. Non-adherent peripheral blood mononuclear cells (PBMC) of healthy adults lysed HUT cells chronically infected with the IIIB or WMJ1 strains of HIV-1 to a significantly greater extent than uninfected HUT cells. In contrast, Sup-T1 cells chronically infected with these two strains of HIV-1 were not lysed to a greater extent than uninfected Sup-T1 cells. Clone A1.25-infected Sup-T1 (A1.25/ Sup-T1), derived from IIIB-infected Sup-T1 cells (IIIB/Sup-T1), were susceptible to non-adherent PBMC-mediated lysis, as were A1.25-infected HUT cells (A1.25/HUT). When non-adherent PBMC were depleted of CD16 (Leu-11b)$^+$ NK cells by treatment with anti-Leu-11b plus C, lysis of HIV-1-infected HUT or Sup-T1 cells was reduced to low levels, indicating that the lysis was mediated by NK cells. Expression of HIV antigens on these target cells did not correlate with their susceptibility to NK cell-mediated lysis. Depletion of interferon-alpha (IFN-α) producing HLA-DR$^+$ cells from non-adherent PBMC had no effect on the magnitude of NK cell-mediated lysis of IIIB or WMJ1-infected HUT cells. In contrast, lysis of A1.25/Sup-T1 or A1.25/HUT cells required the presence of HLA-DR$^+$ cells. IFN-α production appeared to be required for NK cell-mediated lysis of A1.25/Sup-T1 or A1.25/HUT cells, while lysis of HUT cells infected with the WMJ1 or IIIB strains of HIV-1 was IFN-α independent. These results indicate considerable variability in the susceptibility of different HIV-1 infected T cell lines to NK cell-mediated lysis and suggest the existence of alternative mechanisms of activation of NK cells for lysis of HIV-1-infected T cell lines.

Keywords natural killer HIV accessory cells

INTRODUCTION

Most individuals who become infected with the human immu-nodeficiency virus (HIV) do not become symptomatic for several years. This asymptomatic phase of infection may depend at least in part on immune mechanisms which limit the extent of virus replication. However, in the majority of individuals immune mechanisms are unable to prevent progression of HIV infection to disease, namely AIDS, although some seropositive individuals have remained asymptomatic for many years. Further understanding of the immune mechanisms operative during the early asymptomatic stages of infection may suggest ways of enhancing mechanisms which contribute to control of infection.

At some point in the course of infection, prior to the development of symptoms, HIV-infected cells, including cells

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that participate in immune responses and in particular $CD4^+$ lymphocytes, are destroyed, possibly by immune mechanisms. The resultant immunodepression may allow further replication of HIV and is associated with an increased susceptibility to opportunistic infections. Such immunodepression might be prevented by blocking immune mechanisms which contribute to depletion of $CD4^+$ cells.

Only a few studies have examined immune mechanisms capable of eliminating HIV-infected cells. In one such study, HIV-infected peripheral blood $CD4^+$ T cells were shown to be lysed by purifed large granular lymphocytes, a cell population known to contain natural killer (NK) cells (Ruscetti *et al.*, 1986). In another study, antibody-dependent cellular cytotoxicity (ADCC) against $CD4^+$ T cells coated with purified HIV envelope glycoprotein 120 (gp120) was described (Lyerly *et al.*, 1987). Other investigators have shown that cytotoxic T lympho-cytes (CTL) lyse Epstein–Barr virus-transformed B cell lines infected with recombinant HIV–vaccinia viruses (Walker *et al.*, 1987), activated $CD4^+$ T cells exposed to gp120 (Siliciano *et al.*, 1988), and alveolar macrophages obtained from seropositive individuals by bronchoalveolar lavage (Plata *et al.*, 1987). Using

a different approach, Walker *et al.* (1986) showed that CD8^{+} T lymphocytes suppress HIV replication in cultures of peripheral blood mononuclear cells (PBMC).

In the present report we focused on NK cell-mediated lysis of HIV-infected T cell lines. Our interest in NK cells stems from observations indicating a role for such cells in recovery from a number of virus infections (Herberman, 1981; Trinchieri & Perussia, 1984; Fitzgerald, Mendelsohn & Lopez, 1985; Welsh, 1986). Also, NK cells have been shown to limit the replication of at least one virus, herpes simplex virus type 1 (Fitzgerald *et al.*, 1985). We compared the susceptibility of different CD4^{+} T cell lines and strains of HIV-1 to NK cell-mediated lysis. In addition, we determined whether NK cell-mediated lysis of HIV-1-infected targets required interferon-alpha (IFN-α) producing HLA-DR^{+} accessory cells, since we showed previously that NK cell-mediated cytotoxicity against herpes virus-infected targets required HLA-DR^{+} cells in addition to cluster of differentiation (CD)16 antigen-positive NK cells (Bandyopadhyay *et al.*, 1986; Oh *et al.*, 1987).

MATERIALS AND METHODS

Preparation of non-adherent PBMC

Mononuclear cells were obtained from heparinized venous blood of healthy adult volunteers by Ficoll–Hypaque (Pharmacia, Piscataway, NJ) density gradient centrifugation. Adherent cells were removed by incubation on fetal bovine serum (FBS) coated plastic Petri dishes for 1 h at 37°C as previously described (Kumagai *et al.*, 1979). Non-adherent PBMC were used in all experiments. Their viability, as determined by trypan blue dye exclusion, was always >98%.

Antibody plus complement (C) mediated lysis

Negatively selected Leu-11b^{−} and HLA-DR[−] cell populations were obtained as described previously (Bandyopadhyay *et al.*, 1986). Briefly, 5×10^6 non-adherent PBMC in a total volume of 1 ml of RPMI 1640 (GIBCO, Grand Island, NY) containing 10% FBS were incubated for 1 h at room temperature with monoclonal antibody (MoAb) anti-Leu-11b, which recognizes the Fc receptor (CD16) on NK cells (Perussia *et al.*, 1984) (Becton Dickinson, Mountain View, CA) at a final dilution of 1:200; or with MoAb B33.1, which recognizes a non-polymorphic determinant of the HLA-DR molecule (Perussia *et al.*, 1982) (provided by Giorgio Trinchieri, Wistar Institute, Philadelphia, PA) at a final dilution of 1:100. The cells were then centrifuged (100 *g* for 10 min), resuspended in 1 ml of 1:2 diluted baby rabbit C (Cederlane Laboratories, Ontario, Canada) and incubated for 1 h at room temperature. The cells were centrifuged, the supernatant removed, and the treatment with C was repeated. The cells were then washed twice and resuspended in RPMI 1640 containing 10% FBS. Treatment with C alone had no effect on cell viability as determined by trypan blue exclusion and reduced the ability of non-adherent PBMC to mediate killing of HIV-1-infected targets by <15%. To determine the adequacy of depletion, aliquots of 1×10^6 non-adherent PBMC treated with C alone, or with anti-Leu-11b or B33.1 plus C were incubated at 4°C for 30 min with directly conjugated MoAbs different from anti-Leu-11b or B33.1, but of the same specificities, and analysed by flow cytometry as described previously

(Bandyopadhyay *et al.*, 1986). Reactivity of depleted cells with corresponding conjugated MoAb was always less than 5% of that of undepleted PBMC.

Preparation of target cells

Uninfected HUT and Sup-T1 CD4^{+} T cell lines were maintained in RPMI 1640 containing 20% or 10% FBS, respectively, and passaged twice weekly. These cell lines were chronically infected with the IIIB (Hoxie *et al.*, 1986) or WMJ1 (Starcich *et al.*, 1986) strains of HIV-1 as described previously (Hoxie *et al.*, 1986). Briefly, uninfected HUT or Sup-T1 cells were inoculated with cell-free IIIB or WMJ1 virus equivalent to 300 000 reverse transcriptase units and incubated at 37°C. Chronically infected cultures were established from cells that survived the initial infection. The A1.25 clone (A1.25/Sup-T1) was derived from IIIB-infected Sup-T1 cells (IIIB/Sup-T1) by limiting dilution. This clone was originally selected for its high level of CD4 expression as determined by immunofluorescent staining with OKT4 MoAb. Although HIV infection has been shown to induce down modulation of CD4 from the cell surface, the A1.25 clone showed strong reaction with OKT4 but not with OKT4A. A1.25/Sup-T1 cells were used in the present experiments because these cells also expressed high levels of surface HIV antigens (see below). A1.25-infected HUT cells (A1.25/HUT) were prepared by inoculating uninfected HUT cells with cell-free virus obtained from A1.25/Sup-T1 cells. All target cells were tested for mycoplasma contamination by Hoechst staining at regular intervals, and all were repeatedly negative.

Detection of surface antigens

The presence of HIV antigens on the surface of infected cells was documented by indirect immunofluorescence (Hoxie *et al.*, 1985). Briefly, infected and uninfected cells were incubated for 30 min at 4°C with HIV-seropositive or seronegative human serum diluted 1:20, the optimal dilution as determined in preliminary studies. The seropositive serum reacted with major proteins of HIV by Western blot analysis. After incubation, the cells were washed three times with phosphate-buffered saline (PBS) at 4°C, then incubated with FITC-conjugated goat anti-human IgG at 4°C for 30 min. After three washes the cells were fixed with 4% paraformaldehyde for 16 h and analysed using an Epics C fluorescence-activated flow cytometer (Coulter Electronics, Hialeah, FL) equipped with an u.v.-enhanced 5 W argon laser. The laser was operated at 300 mW output at a wave length of 488 nm. The forward angle diode was set at a gain of 10 and the right angle photomultiplier tube was set at a gain of 5 and voltage of 475. The fluorescence photomultiplier tube was set at varying voltages dependent on specific experimental conditions. Individual cells were electronically gated to exclude aggregates from evaluation. In all, 5000 events were accumulated for each sample with fluorescence recorded in a log mode in 256 channels. Cells were considered positive when their fluorescence intensity exceeded the threshold at which 99% of unstained cells had lower fluorescence intensity. A comparison of the relative density of HIV antigens present on the surface of infected target cells were made by the evaluation of flow cytometric mean fluorescence intensities derived from events accumulated in log mode in 256 channels.

Assays for NK cell-mediated lysis

The effector cells used were non-adherent PBMC; NK cell-

depleted non-adherent PBMC (Leu-11b⁻); HLA-DR-positive cell-depleted (HLA-DR⁻) non-adherent PBMC; and 1:1 mixtures of Leu-11b⁻ and HLA-DR⁻ cells. The HIV-1 infected and uninfected target cells (1–2 × 10⁶ cells) were labelled with 100 μCi of Na$_2$^{51}CrO$_4$ (407 mCi/mg; ICN Radiochemicals, Irvine, CA) for 5–6 h and washed three times with medium. Aliquots of 5 × 10³ labelled target cells were added to round-bottomed microtitre plate wells. Effector cells were then added to give effector-to-target cell (E:T) ratios of 50:1 in a final volume of 0·2 ml. The plates were centrifuged at 100 g for 5 min and then incubated at 37°C in 5% CO$_2$. After 18 h incubation, 0·1 ml of supernatant was harvested from each well without disturbing the cell pellet and counted in a gamma-scintillation counter. In selected experiments, additional 50-μl aliquots of supernatant were harvested from triplicate wells, pooled, and stored at −20°C for subsequent determinations of IFN-α. The percent of ^{51}Cr release was calculated using the formula:

% ^{51}Cr release =

$$\frac{\text{Experimental} - \text{Spontaneous ct/min}}{\text{Total} - \text{Spontaneous ct/min}} \times 100$$

where spontaneous release was that obtained in the absence of effector cells and total release was that obtained from target cells incubated with 1% triton X-100 detergent. Spontaneous release from uninfected and HIV-1-infected targets never exceeded 30% of total release.

IFN assay

A previously described cytopathic effect inhibition assay was used to quantify IFN-α activity in the NK assay supernatants (Bandyopadhyay *et al.*, 1988). Bovine cell line MDBK, which detects human IFN-α but not IFN-γ (Gresser *et al.*, 1974; Arbeit, Leary & Levin, 1982), was used as the indicator cell line. Briefly, two-fold serial dilutions of samples were incubated with MDBK cells in microtitre plates for 24 h at 37°C. The monolayers were then challenged with vesicular stomatitis virus (2500 plaque-forming units (PFU)/well). After further incubation for 24 h at 37°C, the monolayers were stained with 0·5% crystal violet/70% ethanol. Each assay included cell and virus controls. Concentrations were calculated in international units (IU) based on results obtained with the National Institutes of Health reference standard for IFN-α (G-023-901-527).

RESULTS

Characterization of T cell lines chronically infected with HIV-1 strains

Chronically infected T cell lines were prepared as described in Materials and Methods. HIV surface antigens on infected Sup-T1 and HUT cells were detected by cytofluorography (Fig. 1). Uninfected cells showed no fluorescence when reacted with HIV seronegative or seropositive sera (not shown). HIV-infected cells showed minimal fluorescence when reacted with a seronegative serum (Fig. 1). When HIV-infected HUT cells were reacted with HIV seropositive serum, IIIB/HUT demonstrated the greatest frequency of positive cells (92·2%) and the highest mean fluorescence intensity (156·6). Less A1.25/HUT cells were positive (80·7%), but those that were positive demonstrated high fluorescence intensity (143·0). In contrast, only 59·1% of WMJ1/HUT cells were positive, and their mean fluorescence intensity was lower (100·1). Among the Sup-T1-infected cells,
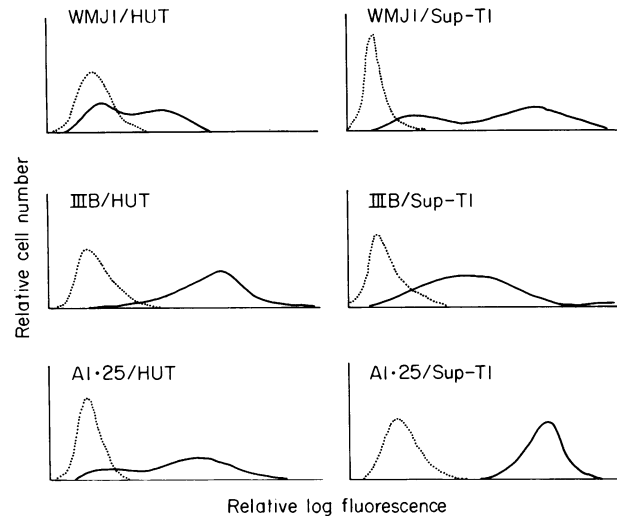

Fig. 1. Indirect immunofluorescence of HIV-infected T cell lines. The methods used for immunofluorescent staining and cytofluorographic analysis are described in Materials and Methods. Results in the presence of HIV-seronegative (·····) and HIV-seropositive (——) sera are shown for each HIV-infected line. The results are representative of three different experiments.

A1.25 clone demonstrated the highest positivity (98·3%) and fluorescence intensity (184·4) when reacted with HIV-seropositive serum. WMJ1/Sup-T1 and IIIB/Sup-T1 were similar with respect to positivity (79·4% and 81·4%, respectively), but the fluorescence intensity was higher for WMJ1/Sup-T1 (169·1 versus 117·1).

Cytotoxicity against HIV-infected T cell lines

We examined the susceptibility of HUT and Sup-T1 cells, chronically infected with different strains of HIV-1, to NK cell-mediated lysis. In preliminary experiments different E:T ratios ranging from 12·5:1 to 100:1 and assay periods ranging from 4 h to 18 h were used. ^{51}Cr release rose linearly with increasing E:T ratios from 12·5:1 to 100:1, and optimal target cell lysis was detected in 18 h assays. All subsequent experiments were done using E:T ratios of 50:1 and 18 h incubation. Sup-T1 cells chronically infected with the WMJ1 or IIIB strains of HIV-1 were not lysed to a greater extent than uninfected Sup-T1 cells by non-adherent PBMC of healthy adults (Table 1). In contrast, the A1.25/Sup-T1 cells, which had been derived from IIIB/Sup-T1 cells, were susceptible to non-adherent PBMC-mediated lysis. Non-adherent PBMC of healthy adults lysed all of the HIV-1-infected HUT cells to a significantly greater extent than uninfected HUT cells.

To identify the cells participating in the killing of HIV-infected targets, effector cell populations were depleted of NK cells or HLA-DR⁺ cells using the anti-CD16 (low-affinity FcR) antibody anti-Leu-11b plus C, or the anti-HLA-DR antibody B33.1 plus C, respectively. Treatment of non-adherent PBMC with anti-Leu-11b plus C significantly reduced the magnitude of lysis of each of the targets compared with results obtained with non-adherent PBMC treated with C alone, indicating that most of the lysis was mediated by NK cells (Table 1). Treatment of non-adherent PBMC with anti-HLA-DR antibody plus C significantly reduced lysis of A1.25/Sup-T1 or A1.25/HUT cells, but not of IIIB/HUT or WMJ1/HUT cells. When Leu-11b⁻

Table 1. Cytotoxicity mediated by non-adherent peripheral blood mononuclear cells (PBMC), Leu-11b⁻ and HLA-DR⁻ cells against Sup-T1 and HUT cells chronically infected with different strains of HIV-1

Effector cells in natural killer assays	No. of experiments	⁵¹Cr Release from target cells (%)			
		Uninfected Sup-T1	WMJ1/Sup-T1	IIIB/Sup-T1	A1.25/Sup-T1
C′ treated PBMC	4	22·7±5·6	24·3±5·6	23·7±9·8	52·2±10·3*
Leu-11b⁻	4	8·3±3·5†	7·6±3·0†	5·0±2·0†	8·5±2·0†
HLA-DR⁻	4	25·1±6·3	22·2±8·9	20·6±5·7	13·3±3·7†
Leu-11b⁻+HLA-DR⁻	4	22·1±1·6	23·3±3·7	21·4±7·6	50·3±8·4
		Uninfected HUT	WMJ1/HUT	IIIB/HUT	A1.25/HUT
C′ treated PBMC	6	26·6±6·1	73·3±5·8‡	71·7±13·3‡	50·5±8·0‡
Leu-11b⁻	4	6·0±7·2†	17·2±6·2†	19·4±8·9†	11·5±3·1†
HLA-DR⁻	4	27·6±16·8	65·3±0·5	68·2±8·2	23·6±7·6§
Leu-11b⁻+HLA-DR⁻	4	26·0±13·4	74·8±1·2	71·5±9·2	35·6±11·5

Mean±s.d.
⁵¹Cr release was assayed as described in Materials and Methods; effector-to-target ratio, 50:1.
Leu-11b⁻ and HLA-DR⁻ cells were prepared as described in Materials and Methods.
Equal numbers of Leu-11b⁻ and HLA-DR⁻ cells were mixed and added to NK assays at a final effector-to-target ratio of 100:1.
* $P<0.05$ compared with uninfected targets; † $P<0.02$ compared with results with PBMC; ‡ $P<0.02$ compared with uninfected targets; § $P<0.05$ compared with results with PBMC. (Student's t-test).

Table 2. Interferon-alpha (IFN-α) concentrations in supernatants of natural killer NK assays containing various targets

Effector cells in NK Assays	Target cells			
	IIIB/HUT	WMJ1/HUT	A1.25/HUT	A1.25/Sup-T1
None	<0·7	<0·7	<0·7	<0·7
C treated PBMC	118·8	118·8	94·7	118·7
HLA-DR	<14·8	<14·8	<0·7	<29·6

Supernatants were harvested and tested for IFN-α as described in Materials and Methods.
Effector cells were incubated with target cells at a ratio of 50:1 for 18 h at 37°C.
HLA-DR⁻ cells were prepared as described in Materials and Methods.
Results shown are representative of three experiments with similar results.

cells (NK depleted) were mixed with HLA-DR⁻ cells (HLA-DR⁺ cell depleted) at a ratio of 1:1, NK activity against A1.25/Sup-T1 was almost completely restored and activity against A1.25/HUT cells was partially restored. Thus HLA-DR⁺ accessory cells were required for NK cell-mediated lysis of A1.25/Sup-T1 or A1.25/HUT cells, but not of IIIB/HUT or WMJ1/HUT cells.

Role of IFN-α in NK activity against HIV-infected targets
In order to determine whether production of IFN-α contributed to NK cell-mediated lysis of HIV-1-infected targets, superna-

tants harvested at the end of the NK assays reported above were assayed for IFN-α. Supernatants of uninfected or HIV-1-infected HUT or Sup-T1 cells alone or of non-adherent PBMC incubated with uninfected HUT or Sup-T1 cells did not contain detectable IFN-α (results not shown). Non-adherent PBMC incubated with IIIB/HUT, WMJ1/HUT or A1.25/HUT produced substantial amounts of IFN-α (Table 2). In contrast, non-adherent PBMC incubated with NK-resistant WMJ1/Sup-T1 or IIIB/Sup-T1 cells did not produce detectable amounts of IFN-α (results not shown). When HLA-DR⁻ effector cells were incubated with each of these HIV-1-infected targets, IFN-α was not produced (Table 2). Thus, IFN-α producing HLA-DR⁺ cells appeared to be required for NK cell-mediated lysis of A1.25/Sup-T1 and A1.25/HUT cells, but not for lysis of IIIB- or WMJ1-infected HUT cells.

DISCUSSION

Our results indicate considerable variability in the susceptibility of different HIV-1-infected targets to NK cell-mediated lysis. NK cells of normal adults lysed HUT cells infected with the IIIB or WMJ1 strains of HIV-1 to a greater extent than uninfected HUT cells. The magnitude of lysis was higher than that reported previously for lysis of HIV-infected peripheral blood CD4⁺ T cells (Ruscetti *et al.*, 1986). This difference may be attributed to the longer incubation periods used for the cytotoxicity assays in the present study. In contrast to our results with HUT cells, Sup-T1 cells infected with the IIIB or WMJ1 strains of HIV-1, were not lysed to a greater extent than uninfected Sup-T1 cells. Thus, with these HIV-1 strains, susceptibility to lysis depended on the cell line used. Similar variability in susceptibility of different

target cells to NK cell-mediated lysis was reported by Malkovsky *et al.* (1988).

We considered the possibility that differences between HUT and Sup-T1 cells may account for their unequal susceptibility to NK cell-mediated lysis after HIV infection. HUT cells were originally derived from a patient with cutaneous T cell lymphoma. Phenotypically, HUT cells are $CD3^+$, $CD4^+$, and $HLA-DR^+$ (Getchell *et al.*, 1986). It has been reported that HUT cells secrete small amounts of interleukin-2 (IL-2) (Gootenberg *et al.*, 1981), and IL-2 is a potent stimulator of NK cells. However, supernatants of the HIV-infected and uninfected HUT cells did not stimulate NK cells to lyse target cells (Bandyopadhyay *et al.*, unpublished observations). Thus, secretion of IL-2 by HUT cells could not explain their susceptibility to lysis. Sup-T1 cells were derived from a patient with non-Hodgkin's lymphoma. Their phenotype is $CD3^-$, $CD4^+$, and $HLA-DR^-$ (Kozbor *et al.*, 1987). These properties of the two cell lines could not explain their unequal susceptibility to NK cell-mediated lysis, and accordingly a series of experiments was carried out on possible mechanisms involved.

To determine whether resistance to NK cell-mediated lysis was an intrinsic property of Sup-T1 cells, we tested a clone of IIIB-infected Sup-T1 cells, A1.25/Sup-T1, that expressed large amounts of HIV antigens at the cell surface. This cell line was susceptible to NK cell-mediated lysis, suggesting that A1.25/Sup-T1 contains biological variants of the parent IIIB strain, and that these variants are responsible for the increased susceptibility to lysis.

For HIV-1-infected Sup-T1 cells there appeared to be a relationship between expression of HIV antigens at the cell surface and susceptibility to NK cell-mediated lysis, since HIV antigens were expressed on a higher percentage of A1.25/Sup-T1 cells compared to the other HIV-1-infected Sup-T1 cells, and the intensity of fluorescence was highest on A1.25/Sup-T1 cells. However, among the HIV-1-infected HUT cells no such relation existed. WMJ1/HUT cells were as susceptible to lysis as the other HIV-1-infected cells, although fewer WMJ1/HUT expressed HIV antigens, and the intensity of the fluorescence was lower. Similarly, when results with the two cell lines were considered together, no apparent relation was found between HIV antigen expression and susceptibility to lysis. Most notably, WMJ1/Sup-T1 expressed HIV antigens to a greater extent than WMJ1/HUT, but only the latter were susceptible to NK cell-mediated lysis. These findings are similar to our previous observation that susceptibility of cytomegalovirus (CMV) infected targets to NK cell-mediated lysis was independent of cell surface CMV late antigen expression (Bandyopadhyay *et al.*, 1988). These observations suggest that virally induced molecules other than serologically detected viral antigens are involved in susceptibility to NK cell-mediated lysis.

We reported previously that NK cell-mediated lysis of herpes virus-infected targets requires the presence of IFN-α producing $HLA-DR^+$ accessory cells that do not bear surface markers of B cells, T cells, NK cells, or monocytes (Bandyopadhyay *et al.*, 1986; Oh *et al.*, 1987). In the present study, lysis of WMJ1 or IIIB-infected HUT cells did not require $HLA-DR^+$ accessory cells. In contrast, lysis of A1.25/HUT or A1.25/Sup-T1 cells did require the presence of these accessory cells. These observations support the idea that A1.25-infected cells contain biological variants of the IIIB strain of HIV-1, and that such variants are responsible for this difference.

Since the accessory role of $HLA-DR^+$ cells for natural killing of CMV-infected targets appeared to involve IFN-α production, we examined the production of IFN-α when nonadherent PBMC or HLA-DR-depleted non-adherent PBMC were incubated with HIV-infected targets. Our results indicate that substantial amounts of IFN-α were produced when nonadherent PBMC were incubated with chronically infected T cells that were susceptible to NK cell-mediated lysis (Table 2). Depletion experiments indicated that the producer cell was $HLA-DR^+$. In contrast to results with NK susceptible targets, IFN-α was not detected when non-adherent PBMC were incubated with the NK-resistant targets WMJ1/Sup-T1 or IIIB/Sup-T1. Thus, the inability of these latter targets to induce IFN-α production may explain the failure to detect NK activity against them. Production of IFN-α did not correlate with the frequency or intensity of HIV antigen expression on target cells.

Although IFN-α was produced during interaction of nonadherent PBMC with NK-susceptible targets, its presence was not always required for lysis to occur. Non-adherent PBMC depleted of $HLA-DR^+$ cells efficiently lysed WMJ1/HUT or IIIB/HUT, although IFN-α was not detected in corresponding supernatants. In contrast, after depletion of IFN-α producing $HLA-DR^+$ cells, lysis of A1.25/HUT and A1.25/Sup-T1 was significantly reduced. Thus, IFN-α produced by $HLA-DR^+$ cells appeared to be required for maximal NK cell-mediated lysis of A1.25-infected cells, but was not required for lysis of other HIV-infected targets.

Our results indicate that there are important differences among HIV strains and/or biological variants with respect to their ability to render T cell lines susceptible to NK cell-mediated lysis. The mechanisms involved in these differences remain to be determined. The observation that accessory $HLA-DR^+$ cells were required for lysis of only two of the six HIV-1-infected targets tested suggests the existence of alternative mechanisms of activation of NK cells for lysis of HIV-infected T cell lines.

The biological significance of these results is not known. It is likely that the susceptibility of HIV-infected cells to NK cell-mediated lysis is of importance only during the early phases of infection, when NK activity is still intact. During this phase resistance to NK cell-mediated lysis is one mechanism that might allow HIV-infected cells to persist for long periods of time without being destroyed. The emergence of biological variants during the course of HIV infection might favor either long-term survival of infected cells or alternatively their destruction which would contribute to the immunodepression associated with HIV infection. Unfortunately, owing to the small percentage of infected $CD4^+$ cells in seropositive individuals, it is not possible to test the susceptibility of such cells to NK cell-mediated lysis directly. As an alternative, we are presently testing $CD4^+$ lymphocytes of healthy individuals for susceptibility to NK cell-mediated lysis after incubation with strains of HIV-1 obtained serially from infected patients.

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