Natural Killer (NK) Cell Stimulatory Factor Increases the Cytotoxic Activity of NK Cells from Both Healthy Donors and Human Immunodeficiency Virus-infected Patients

By Jihed Chehimi,* Stuart E. Starr,*‡ Ian Frank,§ Manthrasalam Rengaraju,‡ Stephanie J. Jackson,* Carlos Llanes,* Michiko Kobayashi,‡ Bice Perussia,‡ Deborah Young,¶ Elliot Nickbarg,¶ Stanley F. Wolf,¶ and Giorgio Trinchieri‡

*From the *Division of Infectious Diseases and Immunology, The Children's Hospital of Philadelphia; ‡The Wistar Institute of Anatomy and Biology; the §Department of Medicine, Infectious Diseases Section, University of Pennsylvania, Philadelphia, Pennsylvania 19104; and ¶Genetics Institute, Inc., Boston, Massachusetts 02140*

Summary

Natural killer cell stimulatory factor (NKSF), or interleukin 12 (IL-12), is a heterodimeric lymphokine produced by B cells that has multiple effects on T and NK cell functions. NKSF at concentrations as low as 0.4 pM enhances the spontaneous cytotoxic activity of peripheral blood lymphocytes (PBL) against a variety of tumor-derived target cell lines and virus-infected target cells. The combined treatment of PBL with NKSF and IL-2 results in a less than additive enhancement of cytotoxicity. NKSF enhances the cytotoxic activity of spontaneously cytotoxic $CD16^+CD5^-$ NK cells and does not confer cytotoxic activity to $CD16^-CD5^+$ T cells. PBL from patients infected with human immunodeficiency virus (HIV) have significantly lower cytotoxic activity against tumor-derived target cells and virus-infected target cells than PBL from control healthy donors. Treatment of PBL from HIV-infected patients with NKSF and/or IL-2 results in an increase of NK cell cytotoxicity against both types of target cells to levels similar to or higher than those of untreated PBL from healthy donors. PBL from HIV-infected patients produce interferon γ in response to NKSF and/or IL-2, although at levels 5- or 10-fold lower than those produced by PBL from healthy donors. The multiple biological effects of NKSF, its activity at very low molar concentrations, and its ability to synergize with other physiological stimuli suggest that NKSF/IL-12 is a lymphokine likely to have physiological importance and considerable therapeutic potential.

Natural killer cell stimulatory factor (NKSF)[1] is a heterodimeric lymphokine composed of a heavy (p40) and a light chain (p35) covalently linked (1, 2). The NKSF p40 chain belongs to the recently defined cytokine receptor family of proteins and has structural homology with the cellular receptor for IL-6 (3). NKSF has also recently been described as cytotoxic lymphocyte maturation factor (CLMF); the name IL-12 has been proposed for this lymphokine (4, 5).

NKSF was originally purified from the cell-free supernatant fluid of phorbol diester-activated human B lymphoblastoid cell lines. The two genes coding for the p40 and p35 chains of NKSF have been molecularly cloned; simultaneous transfection of mammalian cells with the two genes is necessary for the production of biologically active NKSF (2, 5). However, B lymphoblastoid-cell lines produce an excess of free P40 chain, in addition to the biologically active heterodimer (1, 4).

NKSF exerts a variety of biological functions on human T and NK cells and possibly on other cell types. In particular, it has been shown to: (*a*) induce IFN-γ production from both T and NK cells and synergize in this effect with IL-2, mitogens, phorbol diesters, anti-CD3 antibodies, and allogeneic antigens (1, 2, 6); (*b*) exert a comitogenic effect on fresh resting T cells together with lectins or phorbol diesters (1, 2); (*c*) mediate a direct mitogenic effect on activated T or NK cell blasts (5, 7, and our unpublished results); (*d*) en-

[1] *Abbreviations used in this paper:* n, natural; NKSF, natural killer cell stimulatory factor.

J. Exp. Med. © The Rockefeller University Press • 0022-1007/92/03/0789/08 $2.00
Volume 175 March 1992 789-796

hance the cytotoxic activity of resting peripheral blood NK cells (1, 2); (*e*) synergize with IL-2 in the generation of LAK cells (4).

NKSF, independently or synergistically with other stimuli, enhances the in vitro responsiveness of both T and NK cells, acting as an immunostimulating lymphokine at the level of proliferation, cytotoxic activity, and production of lymphokines. If NKSF is produced by normal B cells upon antigenic stimulation, it could play an important regulatory role in T-B cell interaction, synergizing with IL-2 and antigens to stimulate T cells during an immune response.

PBL from HIV-infected patients have often a reduced NK cell cytotoxic activity (8–11) and the ability to produce IFN-γ (12–16). These reduced functions of PBL from HIV-infected patients can be explained only in part by a specific depletion of the lymphocyte subsets mediating these functions (16–19) and suggest that T and NK cells in the patients are functionally anergic. Identification of lymphokines with stimulating activity on the lymphocytes of HIV-infected patients has important practical implications. NKSF, alone or in combination with other stimuli, might represent a possible stimulant of several functions of lymphocytes of HIV-infected patients.

In this study, we analyze the enhancing effect of NKSF on the cytotoxic activity of PBL from healthy donors and HIV-infected patients. NKSF, at concentrations as low as 10 pg/ml, rapidly (<4 h) and efficiently enhanced the cytotoxic activity of peripheral blood NK cells against a variety of target cells. The depressed cytotoxic activity of PBL from HIV-infected patients is efficiently enhanced by NKSF against both tumor-derived and virus-infected target cells to levels comparable with those mediated by PBL from control healthy donors. NKSF also induced IFN-γ production from the PBL of HIV-infected patients, although at lower levels than from PBL from healthy donors.

Materials and Methods

Patients. 29 consenting adult patients, positive for HIV antibodies and at various stages of the disease, were enrolled in this study. HIV serology was determined using commercially available assays. All patients were repeatedly positive for HIV antibodies using HIV ELISA, and confirmed by immunofluorescence and Western blot analysis. Patient classification was according to their absolute $CD4^+$ cell counts (<200/mm³, between 200 and 500/mm³, higher than 500/mm³). $CD4^+$ and $CD8^+$ lymphocyte subsets were enumerated by immunofluorescence. Healthy HIV-seronegative donors, matched for age and sex, were included as controls. At the time of the study, none of the patients were receiving lymphokine therapy.

Peripheral Blood Lymphocytes. Peripheral blood was obtained from consenting adult healthy donors and patients using heparin as anticoagulant. PBMC were separated on Ficoll-Hypaque density gradient (Lymphoprep; Nygaard and Co., Oslo, Norway). PBL were obtained from PBMC after partial depletion of monocytes by adherence to plastic surfaces (45 min, 37°C). Positive and negative selection of PBL subsets reacting with mAbs was performed using antiglobulin indirect rosetting and gradient separation, as described (20). Cell preparations obtained by this method are >95% pure. The mAbs used in this study were B36.1 (IgG2b, anti-CD5) and B73.1 (IgG1, anti-CD16).

To test the effect of lymphokines on cytotoxic activity, PBMC, PBL, or PBL subsets were incubated (5×10^6 cells/ml in RPMI 1640/10% FCS 37°C) for the indicated times in the presence or absence of NKSF or other lymphokines. In some experiments, cell-free supernatant fluids were collected from 18-h cultures and analyzed for IFN-γ content by RIA (21).

Cytokines and Cytokine Assays. Human rIL-2 (10^7 U/mg) was kindly provided by Dr. T. Taguchi, (Osaka University and Takeda Chemical Industry, Inc., Osaka, Japan). Four different preparations of NKSF were used in this study. Pure natural (n)NKSF (4×10^6 U/ml) was purified from the cell-free supernatant fluid of this phorbol diester–stimulated RPMI 8866 B lymphoblastoid cell line according to Kobayashi et al. (1). In some experiments, a semipurified nNKSF preparation was used, obtained after a two-step purification utilizing hydroxylapatite and Mono Q ion exchange chromatography. The IFN-γ-inducing and NK cell–enhancing activities of this semipurified preparation were completely blocked by neutralizing polyclonal or monoclonal antibodies prepared against the recombinant 40-kD chain of NKSF (2, and our unpublished results). Two preparations of rNKSF were used: crude supernatant fluid from transfected COS cells (250 U/ml) or purified cytokine from transfected CHO cells (10^7 U/mg) (2).

IFN-γ was measured by RIA as previously described (21). NKSF biological activity was measured in an IFN-γ induction assay (1, 6). Briefly, PBL (10^6 cells/200 μl/well) were incubated (18 h, 37°C) with NKSF dilutions in round-bottomed 96-well microtiter plates (Costar, Cambridge, MA). After incubation, triplicate 50-μl samples of the cell-free supernatants were collected for each well and IFN-γ was measured by RIA. 1 U of NKSF activity is defined as the amount required to induce half-maximal IFN-γ production. To eliminate interexperimental variability in the determination of NKSF units, a laboratory standard of pure nNKSF was used. 1 U NKSF correspond to 3.6 pM of this standard nNKSF preparation (1).

Cell-mediated Cytotoxicity Assays. ^{51}Cr release cytotoxic assays with K562, RDMC, Daudi cells, and human CMV-infected fibroblasts (CMV-FS4) were performed as described previously (1, 22) using as effector cells PBMC or PBL, from patients and healthy donors, pretreated or not with rNKSF or other lymphokines. K562, RDMC, and Daudi cell lines were maintained in RPMI 1640 containing 10% FCS and labeled overnight with $Na_2{}^{51}CrO_4$ (50 μCi/10^6 cells; ICN Radiochemical, Irvine, CA); ^{51}Cr release was measured after 3 or 4 h of incubation.

Human embryonic foreskin fibroblasts (FS4 strain; kindly provided by J. Vilcek, New York Medical Center, New York, NY) were maintained in Eagle's Modified MEM (Flow Laboratories, Inc., McLean, VA) supplemented with 10% heat-inactivated FCS and double vitamins. FS4 cells were used at passage 16–24. Monolayers of FS4 cells in 75-cm² flasks were infected with human CMV strain AD-169 at a multiplicity of infection of ~0.1. When 90% of the cells exhibited cytopathic effect, the monolayers were trypsinized, and the cells were washed and frozen at −120°C until use. Cryopreserved CMV-infected targets were thawed and labeled with $Na_2{}^{51}CrO_4$ (50 μCi 10^6 cells) for 1 h at 37°C, 5% CO_2 with gentle shaking every 15 min. Plates were centrifuged at 100 *g* for 5 min and then incubated for 18 h at 37°C, 5% CO_2. All determinations were in triplicate.

Percent specific ^{51}Cr release was calculated using the formula: percent specific ^{51}Cr release = 100× [(cpm experimental − cpm spontaneous)/(cpm maximum − cpm spontaneous)], where spontaneous release was that obtained from target cells incubated with medium alone, and maximum release was that obtained from target cells incubated with 1% Triton X-100.

Results

Enhancement of NK Cell-mediated Cytotoxicity by NKSF. Pretreatment with NKSF for 18 h enhances the spontaneous cytotoxicity of human PBL against a variety of target cell lines, including K562, Daudi, RDMC (1), Raji, U937, and HL-60 (results not shown). The experiments described in this paper have been performed using as target cells the first three cell lines. Although the three cell lines differ in their sensitivity to NK cells, comparable results have been obtained using each of them and representative experiments with only one target cell line are shown. To identify the cytotoxic effector cells responding to stimulation with NKSF, PBL subsets reacting with mAbs to surface differentiation antigens were separated by indirect rosetting and tested for ability to mediate cytotoxicity. These experiments showed that the increased cytotoxicity of PBL treated with NKSF was mediated mainly by $CD16^+CD5^-$ cells (Fig. 1). The enhancement of NK cell cytotoxicity was observed with doses of NKSF as low as 0.1 U/ml (~0.4 pM) using both natural or recombinant (Fig. 2) NKSF. The simultaneous addition of NKSF and IL-2 resulted in only a negligible increase in the cytotoxic activity induced by optimal doses of NKSF (Fig. 2). However, in the presence of 100 or 1,000 U/ml of IL-2, doses of NKSF as low as 0.1 U/ml were able to increase NK cytotoxicity as efficiently as 2.5 U/ml of NKSF alone (Fig. 2).

Effect of NKSF on PBL from HIV-infected Patients. To determine whether NKSF can modulate NK cell cytotoxic activity of HIV-infected patients, cytotoxicity assays were performed. NK cell cytotoxicity activity of healthy subjects against K562 cells (E/T ratio, 50:1) varies widely (range 9–69%, ^{51}Cr release). PBL of HIV-infected patients mediate, on average, lower cytotoxicity against K562 and CMV-FS4 target cells than that mediated by PBL from a panel of 20 healthy (HIV-seronegative) donors (40.9% vs. 17.5% against K562 target cells [$t = 5.56$, $p < 0.001$] and 26.8% vs. 10.5% against CMV-FS4 target cells [$t = 2.83$, $p < 0.05$]) (Table 1, Figure 3).

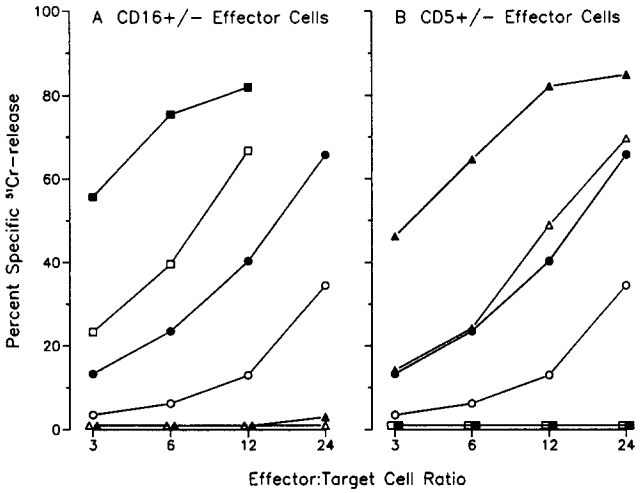

Figure 1. Effect of NKSF on the cytotoxic activity of $CD16^+CD5^-$ NK cells. Total PBL were separated into $CD16^+$ and $CD16^-$, and into $CD5^+$ and $CD5^-$ cells by indirect rosetting. The PBL and the separated subpopulations were incubated (37°C, 18 h, 5 × 10^6 cells/ml) in RPMI 1640 10% FCS in the presence (*filled symbols*) or absence (*open symbols*) of 1 U/ml semipurified NKSF. Cells were then washed and tested as effector cells in a 3-h cytotoxicity assay against ^{51}Cr-labeled RDMC target cells. (*A*) Cytotoxicity of PBL and $CD16^+$ and $CD16^-$ cells separated using antibody B73.1. (*B*) cytotoxicity of PBL and $CD5^+$ and $CD5^-$ cells separated using antibody B36.1. The effector cell preparations were: unseparated PBL (○, ●); antibody-positive cells (□, ■); and antibody-negative cells (△, ▲). This experiment is representative of four with similar results.

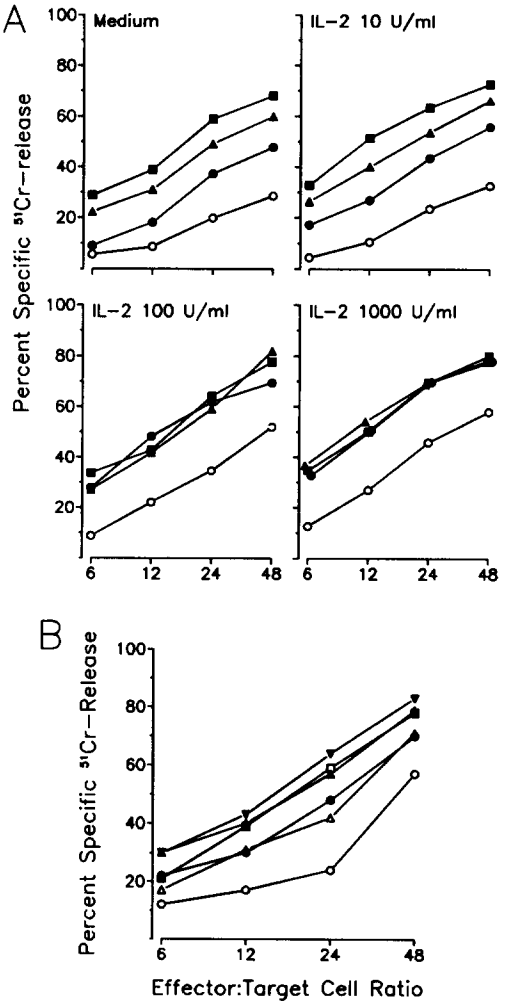

Figure 2. Effects of natural and recombinant NKSF and of IL-2 on the cytotoxic activity of human PBL. (*A*) Fresh PBL were incubated (37°C, 18 h, 5 × 10^6 cells/ml in RPMI 1640 10% FCS) in medium without or with 10, 100, or 1,000 U/ml rIL-2, as indicated, in the absence of NKSF (○) or in the presence of 0.1 U/ml (●); 0.5 U/ml (▲); and 2.5 U/ml (■) semipurified nNKSF. PBL were then washed and tested in a 3-h ^{51}Cr release assay against RDMC cells. This experiment is representative of eight experiments performed using either RDMC or Daudi target cells. (*B*) Fresh PBL were incubated (37°C, 18 h, 5 × 10^6 cells/ml in RPMI 1640 10% FCS) in medium without (○) or with 30 pg/ml (●), 270 pg/ml (▲), 2,430 pg/ml (■) purified CHO rNKSF, or 100 U/ml (△), 500 U/ml (□) rIL-2. PBL were then washed and tested in a 4-h ^{51}Cr release assay against ^{51}Cr-labeled K562 cells. This experiment is representative of four experiments performed with similar results.

Table 1. *Effect of rNKSF and rIL-2 on Cytotoxicity Mediated by PBL against K562 and CMV-FS4 Target Cells*

Target	Donors	$CD4^+/mm^3$	*n*	PBL preincubated with:*			
				Medium	NKSF	IL-2	NKSF + IL-2
K562	Healthy	ND	20	40.9 ± 13.0‡	56.4 ± 11.3	58.2 ± 11.7	61.3 ± 10.0
	HIV infected	All donors	29	17.5 ± 15.5	40.7 ± 14.6	37.1 ± 14.0	49.7 ± 15.5
		<200	16	16.3 ± 16.0	44.6 ± 13.0	36.0 ± 13.5	51.0 ± 14.0
		200–500	9	18.6 ± 9.9	33.0 ± 12.7	35.0 ± 9.3	41.4 ± 14.0
		>500	4	29.3 ± 15.6	46.3 ± 15.5	45.3 ± 15.5	54.0 ± 14.0
CMV-FS4	Healthy	ND	6	26.6 ± 12.0	43.6 ± 11.0	55.6 ± 9.0	60.5 ± 14.0
	HIV infected	All donors	6	10.5 ± 7.0	27.0 ± 7.0	23.0 ± 8.0	38.8 ± 11.0
		<200	5	7.6 ± 3.9	24.8 ± 5.5	22.8 ± 9.0	39.4 ± 12.0
		>500	1	25	39	26	36

* PBL were preincubated (18 h, 37°C) with 1 U/ml rNKSF (COS cell derived), 100 U/ml rIL-2, or both cytokines. After washing, PBL were used as effector cells in a 4-h ^{51}Cr release assay against K562 target cells or in an 18-h ^{51}Cr release against CMV-FS4 target cells.
‡ Mean percent specific ^{51}Cr-release ± SD (E/T ratio, 50:1) of *n* donors tested.

The cytotoxic activity mediated by PBL from HIV-infected patients against K562 was significantly increased by incubation with 1 U/ml of rNKSF ($t = 5.968$, $p < 0.001$) to values comparable with those observed with untreated PBL from control healthy donors, although still significantly lower ($t = 4.06$, $p < 0.001$) than those mediated by NKSF-treated PBL from healthy donors. Killing of CMV-FS4 was significantly increased after preincubation of PBL from HIV-infected patients with 1 U/ml of NKSF ($t = 4.08$, $p < 0.01$). The dose of 1 U/ml of NKSF was as efficient as 100 U/ml of IL-2 in enhancing the cytotoxicity from HIV-infected patients against K562 as well as CMV-FS4 target cells. Treatment of PBL with both NKSF and IL-2 resulted in a higher, but less than additive, enhancement of cytotoxicity when compared with treatments with each cytokine alone (Table 1). When K562 target cells were used, there was no significant difference in response between patients with $CD4^+$ cell counts $<200/mm^3$, and those having $CD4^+$ cell counts between 200 and $500/mm^3$. The number of patients tested, using CMV-FS4 target cells, was too low to evaluate the differences among the three groups.

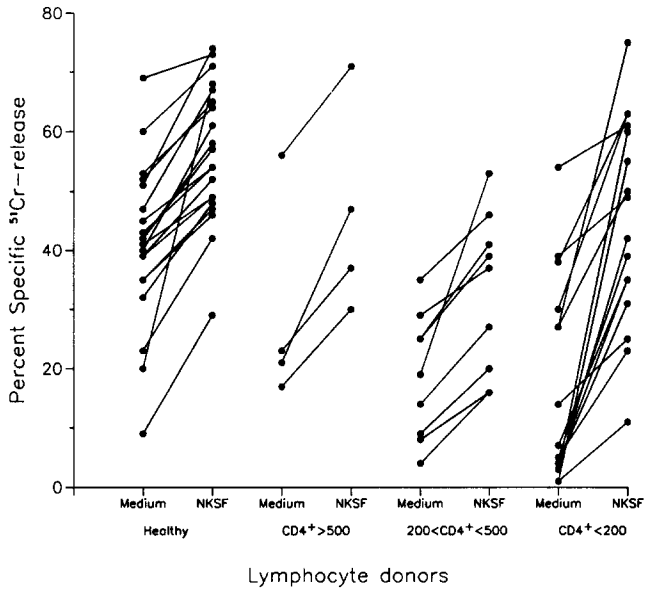

Figure 3. Effect of NKSF on the cytotoxic activity of PBL from healthy (HIV-seronegative) donors and HIV-infected patients. PBL were incubated (37°C, 18 h, 5 × 10^6 cells/ml) in RPMI 1640 10% FCS with or without 1 U/ml rNKSF (COS cell supernatant). Cells were then washed and tested at an E/T cell ratio of 50:1 against ^{51}Cr-labeled K562 in a 4-h cytotoxicity assay. HIV-infected donors were grouped according to the absolute number of $CD4^+$ cells/mm^3. Each pair of symbols represents a different donor. In each experiment, a similar number of healthy controls and HIV-infected patients was analyzed. Healthy controls were matched for age and sex with the HIV-infected patients.

The ability of PBL from nine healthy subjects, and 12 patients already tested for cytotoxicity, to produce IFN-γ in response to NKSF and IL-2 was compared. Stimulation with 100 U/ml of IL-2 or with 1 U/ml of NKSF for 18 h resulted in an IFN-γ production from PBL of HIV-infected patients that was 5- to 10-fold lower than that from PBL of control healthy donors (Table 2). Although simultaneous stimulation of PBL from HIV-infected patients with NKSF and IL-2 induced a mean titer of 153 U/ml, this amount was approximately sixfold lower than that observed after stimulation of PBL from healthy subjects (Table 2). Patients with $CD4^+$ cell counts $>500/mm^3$ produced higher amounts of IFN-γ than those with lower $CD4^+$ cell counts. In the three patients' groups tested, simultaneous stimulation with NKSF and IL-2 induced higher IFN-γ production than that induced by each cytokine alone (Table 2).

Table 2. *Production of IFN-γ by PBL from Healthy Controls and HIV-infected Patients Induced by NKSF and/or IL-2*

Donors	$CD4^+/mm^3$	*n*	PBL incubated with:*			
			Medium	NKSF	IL-2	NKSF + IL-2
			U/ml IFN-γ			
Healthy	ND	9	2.6 ± 2.1‡	92.3 ± 101.0	99.8 ± 119.0	895.5 ± 236.0
HIV infected	All donors	12	0.3 ± 0.6	9.9 ± 9.4	18.6 ± 23.6	153.7 ± 118
HIV infected	<200	5	<0.3	4.4 ± 4.0	2.1 ± 4.0	86.6 ± 58.0
HIV infected	200–500	4	<0.3	6.0 ± 2.5	10.5 ± 8.0	129.5 ± 44.3
HIV infected	>500	3	1.0 ± 0.8	24.3 ± 5.5	57.0 ± 9.9	298.0 ± 135.0

* IFN-γ concentration was measured by RIA in the cell-free supernatant fluid collected from PBL cultured (18 h, 37°C) with or without rNKSF (COS cell derived), 1 U/ml; rIL-2, 100 U/ml; or both cytokines.
‡ Mean ± SD of *n* donors.

Discussion

IFN-α and IFN-β were the first cytokines shown to enhance NK cell–mediated cytotoxic activity (23). Later, IL-2 and TNF were also shown to be stimulatory (24–26). IFN- and IL-2-induced enhancement of cytotoxicity occurs with fast kinetics (a few hours to 1–2 d), and in the absence of cell division (25). In these conditions, IFN and IL-2 increase the cytotoxic activity of the NK cell subset, mostly $CD16^+CD56^+CD3^-$ lymphocytes, and do not induce cytotoxic activity in the majority of $CD3^+$ T cells, which do not mediate spontaneous cytotoxicity (25).

Similarly to IFN-α and IL-2, NKSF is a potent stimulator of NK cell cytotoxic functions of PBL, acting with a rapid kinetics (<4 h) and at extremely low concentrations. Our data show that in an 18-h incubation period, NKSF increases the cytotoxic activity of $CD16^+CD5^-$ NK cells, but does not confer this function to $CD16^-CD5^+$ T cells. As we previously reported for natural NKSF (1), and confirm now using pure recombinant cytokine, NKSF efficiently enhances NK cell–mediated cytotoxicity at concentrations <1 pM, whereas similar effects are observed with IL-2 or IFN-α only when these cytokines are used at concentrations at least 100-fold higher. Because NKSF synergizes with IL-2 to induce IFN-γ production from both T and NK cells (1, 6), it was of interest to assay whether the two lymphokines synergize in enhancing cytotoxic activity. PBL stimulated with optimal concentrations of both IL-2 and NKSF mediated only marginally higher cytotoxicity than PBL stimulated with NKSF alone. However, in the presence of 100 or 1,000 U/ml of IL-2, a maximal stimulation of cytotoxic activity was obtained with concentrations of NKSF (0.1 U/ml or 0.35 pM) that by themselves induce only a modest enhancement.

As previously observed after stimulation with IFN-α or IL-2 (20, 25), only the cytotoxicity of $CD16^+CD5^-$ NK cells is enhanced by NKSF and no cytotoxic ability is induced in the $CD5^+$ T cells at the time points tested. Gubler et al. (5) have described a strong synergistic activity of NKSF and IL-2 in inducing generation of cytotoxic cells against Daudi target cells in a 4-d culture. Whether these activated cytotoxic cells are mostly composed of $CD16^+$ NK cells, as observed in other systems of LAK cell generation, or contain a significant proportion of non-MHC-restricted T cells, remains to be determined.

Infection with HIV is associated with a wide spectrum of clinical stages ranging from asymptomatic illness to full-blown AIDS. In AIDS patients, a multitude of profound defects in the various compartments of the immune system have been described (27). Some of the dysfunctions include a depression in $CD4^+$ T lymphocytes number and function, and a decrease in monocyte functions, NK cell cytotoxic activity, lymphoproliferative responses to mitogens and alloantigens, MHC class II antigen expression, and polyclonal B cell activation (27). In vitro, the production of lymphokines such as IFN-α, IFN-γ, and IL-2 is diminished (12–16, 28). The nature and role of humoral and cellular immune response mechanisms against HIV have not been fully characterized. It is increasingly evident that in vivo progression of AIDS is intimately related with abnormal functioning of the immune system. Tremendous efforts are being made to develop new effective immunomodulatory agents that, in association with antiretroviral drugs, may be of beneficial therapy. Several groups have previously used IL-2 alone in HIV-infected patients with varying results, and other groups are currently studying the effects of AZT and IL-2 in individuals infected with HIV (29–32). However, IL-2 at doses between 1 and 12×10^6 U/m^2 can cause serious side effects. One approach to improving the efficacy of IL-2 therapy while reducing its toxicity is to use a combination of cytokines or a combination of a cytokine with antiretroviral drugs.

Conflicting data have been reported concerning NK cell cytotoxic activity in patients with AIDS (33), although most studies have reported NK cell defects, the pathogenesis of which is still unknown. We have previously reported data on the susceptibility of clonal and polyclonal populations of NK cells to HIV infection, suggesting that loss of NK cells and reduced NK cell functions in patients with AIDS occur via two different mechanisms (22). In the present study, we

evaluated the in vitro effect of NKSF and IL-2 on NK cell–mediated activity and on IFN-γ production by PBL from HIV-infected patients. Since the mechanisms involved in killing of virus-infected targets are different from those involved in lysis of tumor cells, we used K562 cells and CMV-infected fibroblasts, CMV-FS4, as target cells. Our results indicate that HIV-infected patients showed a deficiency in NK cell–mediated cytotoxicity against both target cells, which is in agreement with other studies (33). We did not find a clear correlation between the level of cytotoxicity and the absolute number of $CD4^+$ T lymphocytes. Using NKSF at a concentration of 1 U/ml, our data revealed an increase in NK cell–mediated cytotoxicity in all HIV-infected patients tested. A similar effect was achieved with 100 U/ml of IL-2. Of interest, NKSF restores NK activity of HIV-infected patients to a level similar to that observed with healthy subjects. NKSF-dependent enhancement of NK cell cytotoxicity in normal donors and in patients was completely blocked by two different neutralizing mAbs to rNKSF (not shown). Depletion of $CD16^+$ NK cells from NKSF-stimulated PBL of patients or controls reduced the cytotoxicity against K562 and CMV-FS4 to baseline levels (not shown).

When IL-2 and NKSF were used separately, the levels of IFN-γ production from 12 HIV-infected patients tested were 5- to 10-fold lower than those of healthy donors; stimulation with both cytokines synergistically induced IFN-γ at levels higher than those observed in healthy donors with each cytokine alone. IFN-γ production in patients with AIDS correlated with the absolute $CD4^+$ cell number.

Clinical trials using IFN-γ in patients with AIDS (34, 35) have been initiated on the basis of the observation that although the patients have a defect in their ability to produce IFN-γ in response to different inducers, their PBMC were responsive to IFN-γ in vitro (12, 36–38). Heagy et al. (35) reported a phase I/II study in which 21 AIDS patients were treated with IFN-γ. In six patients, a decrease in p24 core antigen was observed, suggesting an antiviral effect of IFN-γ. In patients with high $CD4^+$ cell counts, a transient regression of the malignant lesions associated with Kaposi sarcomas was also observed. Murphy et al. (34) reported that IFN-γ-treated patients have fewer nonopportunistic infections than the IL-2-treated ones.

It is clear that the treatment of a syndrome with multiple immunological dysfunctions such as AIDS would be facilitated by the combined use of multiple cytokines and antiviral agents that would offer the possibility to correct or compensate some of the dysfunctions while exerting a direct antiviral effect. NKSF is one such potential candidate cytokine because of its multiple immunopotentiating effects, its activity at much lower concentrations than other cytokines, and, particularly important, its ability to synergize with other physiological stimuli on immune cells, such as IL-2 or antigenic stimulation.

This work was supported in part by U.S. Public Health Service grants CA-10815, CA-20833, CA-32898, CA-37155, CA-40256, CA-45284, and CA-47589, and by the Brandywine Research Foundation. B. Perussia is a scholar of the Leukemia Society of America.

Address correspondence to Giorgio Trinchieri, The Wistar Institute, 3601 Spruce Street, Philadelphia, PA 19104. M. Kobayashi's present address is Genetics Institute Inc. of Japan, 1-10-13 Higashi Azabu, Minato-ku, Tokyo 106, Japan. B. Perussia's present address is the Jefferson Cancer Institute, Department of Microbiology and Immunology, Thomas Jefferson University, Philadelphia, PA 19107.

Received for publication 19 September 1991 and in revised form 4 November 1991.

References

1. Kobayashi, M., L. Fitz, M. Ryan, R.M. Hewick, S.C. Clark, S. Chan, R. Loudon, F. Sherman, B. Perussia, and G. Trinchieri. 1989. Identification and purification of natural killer cell stimulatory factor (NKSF), a cytokine with multiple biologic effects on human lymphocytes. *J. Exp. Med.* 170:827.
2. Wolf, S.F., P.A. Temple, M. Kobayashi, D. Young, M. Dicig, L. Lowe, R. Dzialo, L. Fitz, C. Ferenz, R.M. Hewick, K. Kelleher, S.H. Herrmann, S.C. Clark, L. Azzoni, S.H. Chan, G. Trinchieri, and B. Perussia. 1991. Cloning of cDNA for natural killer cell stimulatory factor, a heterodimeric cytokine with multiple biologic effects on T and Natural Killer cells. *J. Immunol.* 146:3074.
3. Gearing, D.P., and D. Cosman. 1991. Homology of the p40 subunit of Natural Killer cell Stimulatory Factor (NKSF) with the extracellular domain of the interleukin-6 receptor. *Cell.* 66:9.
4. Stern, A.S., F.J. Podlaski, J.D. Hulmes, Y.E. Pan, P.M. Quinn, A.G. Wolitzky, P.C. Familletti, D.L. Stremlo, T. Truitt, R. Chizzonite, and M.K. Gately. 1990. Purification to homogeneity and partial characterization of cytotoxic lymphocyte maturation factor from human B-lymphoblastoid cells. *Proc. Natl. Acad. Sci. USA.* 87:6808.
5. Gubler, U., A.O. Chua, D.S. Schoenhaut, C.M. Dwyer, W. McComas, R. Motyka, N. Nabavi, A.G. Wolitzky, P.M. Quinn, P.C. Familletti, and M.K. Gately. 1991. Coexpression of two distinct genes is required to generate secreted bioactive cytotoxic lymphocyte maturation factor. *Proc. Natl. Acad. Sci. USA.* 88:4143.
6. Chan, S.H., B. Perussia, J.W. Gupta, M. Kobayashi, M. Pospisil, H.A. Young, S.F. Wolf, D. Young, S.C. Clark, and G. Trinchieri. 1991. Induction of IFN-γ production by NK cell stimula-

tory factor (NKSF): characterization of the responder cells and synergy with other inducers. *J. Exp. Med.* 173:869.
7. Gately, M.K., B.B. Desai, A.C. Wolitzky, P.M. Quinn, C.M. Dwyer, F.J. Podlaski, P.C. Familletti, F. Sinigaglia, R. Chizounite, V. Gubler, and A.S. Stern. 1991. Regulation of human lymphocyte proliferation by a heterodimeric cytokine, IL-12 (cytotoxic lymphocyte stimulation factor). *J. Immunol.* 147:874.
8. Fontana, L., M.C. Sirianni, G. de Sanctis, M. Carbonari, B. Ensoli, and F. Aliuti. 1986. Deficiency of natural killer activity, but not of natural killer binding, in patients with lymphoadenopathy syndrome positive for antibodies to HTLV-III. *Immunobiology.* 171:425.
9. Bonavida, B., J. Katz, and M. Gottlieb. 1986. Mechanism of defective NK cell activity in patients with acquired immunodeficiency syndrome (AIDS) and AIDS-related complex. I. Defective trigger on NK cells for NKCF production by target cells, and partial restoration by IL-2. *J. Immunol.* 137:1157.
10. Katzman, M., and M.M. Lederman. 1986. Defective post-binding lysis underlies the impaired natural killer activity in factor VIII-treated, human T lymphotropic virus type III seropositive hemophiliacs. *J. Clin. Invest.* 77:1057.
11. Sirianni, M.C., S. Soddus, W. Malorni, G. Arancia, and F. Aiuti. 1988. Mechanisms of defective natural killer cell activity in patients with AIDS is associated with defective distribution of tubulin. *J. Immunol.* 140:2565.
12. Murray, H.W., B.Y. Rubin, H. Masur, and R.B. Roberts. 1984. Impaired production of lymphokines and immune (gamma) interferon in the acquired immunodeficiency syndrome. *N. Engl. J. Med.* 310:883.
13. Ciobanu, N.K., K. Welte, G. Kruger, S. Venuta, J. Gold, S.P. Feldman, C.Y. Wang, B. Koziner, M.A.S. Moore, B. Safai, and R. Mertelsmann. 1983. Defective T cell response to PHA and mitogen monoclonal antibodies in male homosexuals with acquired immunodeficiency syndrome and its *in vitro* correction by interleukin 2. *J. Clin. Immunol.* 3:461.
14. Cauda, R., S.F. Tyring, E. Tamburrini, G. Ventura, M. Tambarello, and L. Ortona. 1988. Diminished interferon gamma production may be the earliest indicator of infection with the human immunodeficiency virus. *Viral Immunol.* 1:247.
15. Rudy, T., G. Opelz, R. Gerlach, V. Daniel, and K. Schimpf. 1988. Correlation of *in vitro* immune defects with impaired gamma interferon response in human immunodeficiency-virus-infected individuals. *Vox Sang.* 54:92.
16. Maggi, E., D. Macchia, P. Parronchi, M. Mazzetti, A. Ravina, D. Milo, and S. Romagnani. 1987. Reduced production of interleukin 2 and interferon-gamma and enhanced helper activity for IgG synthesis by cloned $CD4^+$ T cells from patients with AIDS. *Eur. J. Immunol.* 17:1865.
17. Vuillier, F., N.E. Bianco, L. Montagnier, and G. Dighiero. 1988. Selective depletion of low-density $CD8^+$, $CD16^+$ lymphocytes during HIV infection. *AIDS Res. Hum. Retroviruses.* 4:121.
18. Notka, M.A., and R.B. Pollard. 1990. Patterns of interferon-gamma production by peripheral blood mononuclear cells from patients with human immuno-deficiency virus infection. *J. Interferon Res.* 10:173.
19. Sirianni, M.C., F. Tagliaferri, and F. Aiuti. 1990. Pathogenesis of the natural killer cell deficiency in AIDS. *Immunol. Today.* 11:81.
20. Perussia, B., S. Starr, S. Abraham, V. Fanning, and G. Trinchieri. 1983. Human natural killer cells analyzed by B73.1, a monoclonal antibody blocking Fc receptor functions. I. Characterization of the lymphocyte subset reactive with B73.1. *J. Immunol.* 130:2133.
21. Murphy, M., R. Loudon, M. Kobayashi, and G. Trinchieri. 1986. Gamma interferon and lymphotoxin, released by activated T cells, synergize to inhibit granulocyte-monocyte colony formation. *J. Exp. Med.* 164:263.
22. Chehimi, J., S. Bandyopadhyay, K. Prakash, B. Perussia, N.F. Hassan, H. Kawashima, D. Campbell, J. Kornbluth, and S.E. Starr. 1991. *In vitro* infection of natural killer cells with different human immunodeficiency virus type I isolates. *J. Virol.* 65:1812.
23. Trinchieri, C., D. Santoli, R. Dee, and B.B. Knowles. 1978. Anti-viral activity induced by culturing lymphocytes with tumor-derived or virus-transformed cells. Identification of the anti-viral activity as interferon and characterization of the human effector lymphocyte subpopulation. *J. Exp. Med.* 147:1299.
24. Henney, C.S., K. Kuribayashi, D.E. Kern, and S. Gillis. 1981. Interleukin 2 augments natural killer cell activity. *Nature (Lond.).* 291:335.
25. Trinchieri, G., M. Matsumoto-Kobayshi, S.C. Clark, J. Sheehra, L. London, and B. Perussia. 1984. Response of resting human peripheral blood natural killer cells to interleukin-2. *J. Exp. Med.* 160:1147.
26. Ostensen, M.E., D.L. Thiele, and P.E. Lipsky. 1987. Tumor necrosis factor-alpha enhances cytolytic activity of human natural killer cells. *J. Immunol.* 138:4185.
27. Resenberg, Z.F., and A.S. Fauci. 1989. The immunopathogenesis of HIV infection. *Adv. Immunol.* 47:377.
28. Rossol, S., R. Voth, H.P. Laubenstein, W.E.G. Müller, H.C. Schröder, K.H. Meyer Zum Büschenfelde, and G. Hess. 1989. Interferon production in patients infected with HIV-1. *J. Infect. Dis.* 159:815.
29. Lane, H.C., J.P. Siegel, A.H. Rook, H. Masur, E.P. Gelmann, G.V. Quinnan, and A.S. Fauci. 1984. Use of interleukin-2 in patients with acquired immunodeficiency syndrome. *J. Biol. Response Mod.* 3:512.
30. Mertelsmann, R., K. Welte, C. Sternberg, R. O'Reilly, M.A.S. Moore, B.D. Clarkson, and H.F. Oettgen. 1984. Treatment of immunodeficiency with interleukin-2: initial exploration. *J. Biol. Response Modif.* 4:483.
31. Volberding, P., D.J. Moody, D. Beardslee, E.C. Bradley, and C.B. Wofsy. 1987. Therapy of acquired immune deficiency syndrome with recombinant interleukin-2. *AIDS Res. Hum. Retroviruses.* 3:115.
32. Schwartz, D.H., G. Skowron, and T.C. Merigan. 1991. Safety and effects of interleukin-2 plus zidovudine in asymptomatic individuals infected with human immunodeficiency virus. *J. Acq. Immun. Def. Synd.* 4:11.
33. Brenner, B.G., A. Dascal, R.G. Margolese, and M.A. Wainberg. 1989. Natural killer cell function in patients with acquired immunodeficiency syndrome and related diseases. *J. Leukocyte Biol.* 46:75.
34. Murphy, P.M., C.H. Lane, J.I. Gallin, and A.S. Fauci. 1988. Marked disparity in incidence of bacterial infections in patients with the acquired immunodeficiency syndrome receiving interleukin-2 or interferon-gamma. *Ann. Intern. Med.* 108:36.
35. Heagy, W., J. Groopman, J. Schindler, and R. Finberg. 1990. Use of IFN-γ in patients with AIDS. *J. Acq. Immun. Def. Synd.* 3:584.
36. Murray, H.W., D. Scavuzzo, J.L. Jacobs, M.H. Kaplan, D.M.

Libby, J. Schindler, and R.B. Roberts. 1987. *In vitro* and *in vivo* activation of human mononuclear phagocytes by interferon gamma. Studies with normal and AIDS monocytes. *J. Immunol.* 138:2457.

37. Heagy, W., V.E. Kelley, T.B. Strom, K. Mayer, H.M. Shapiro, R. Mandel, and R.W. Finberg. 1984. Decreased expression of human class II antigens on monocytes from patients with acquired immunodeficiency syndrome. *J. Clin. Invest.* 74:2089.
38. Heagy, W., T.B. Strom, V.E. Kelley, J. Collela, C. Crumpacker, J.M. Williams, H.M. Shapiro, L. Laubenstein, and R. Finberg. 1989. Recombinant human gamma interferon enhances *in vitro* activation of lymphocytes isolated from patients with acquired immunodeficiency syndrome. *Infect. Immun.* 57:3619.