INFECTION AND IMMUNITY, May 1984, p. 339–343
0019-9567/84/050339-05$02.00/0
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Vol. 44, No. 2

Interleukin 2 Augmentation of Natural Killer Cell Activity in Homosexual Men with Acquired Immune Deficiency Syndrome

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Received 19 September 1983/Accepted 26 January 1984

Natural killer cell activity of peripheral blood mononuclear cells against K562 myeloid cells was studied in 8 normal heterosexual men, 5 healthy homosexual men, and 11 homosexual men with acquired immune deficiency syndrome. The overall natural killer cell activity was lower in healthy homosexual men and in homosexual men with acquired immune deficiency syndrome. Peripheral blood mononuclear cells from four normal heterosexual men were preincubated overnight in complete medium supplemented with various concentrations of lectin-free interleukin 2 lacking interferon. Cells from these cultures exhibited a dose-dependent augmentation of natural killer cell activity. Peripheral blood mononuclear cells obtained from 8 normal heterosexuals, 5 healthy homosexuals, and 11 homosexual men with acquired immune deficiency syndrome were preincubated overnight in complete medium supplemented with 20% interleukin 2. Natural killer cell activity cultured in 20% interleukin 2 increased to 78.4 ± 8.4 (mean ± standard deviation) from 30.8 ± 11.0 in normal heterosexual men, to 68.3 ± 17.6 from 16.8 ± 13.6 in healthy homosexual men and to 49.3 ± 15.3 from 11.6 ± 6.1 in homosexual men with acquired immune deficiency syndrome at a 100:1 effector/target cell ratio. These results suggest that interleukin 2 is capable of directly stimulating natural cell-mediated cytotoxicity. Consideration of the use of interleukin 2 as a potential therapeutic agent to modify immune responses in disorders such as acquired immune deficiency syndrome appears warranted.

Natural killer (NK) cells are a recently defined subpopulation of lymphoid cells that are present in most normal persons and are unique in their ability to spontaneously lyse certain immature cells, virus-infected cells, and malignant cells irrespective of major histocompatibility restriction (12). NK cells lack the features of mature T cells, B cells, or macrophages. Most NK cells in human peripheral blood bear Fc receptors, but lack immunoglobulin on the surface. NK cells may play an important role defending the body against malignancy and viral or bacterial infections (12, 22).

Interleukin 2 (IL-2) or T cell growth factor (TCGF), a lymphokine secreted by T cells, has been shown to modulate several in vitro immune responses. It plays an important role in inducing lymphocyte proliferation and differentiation (6). IL-2 has been shown to trigger the clonal expansion of antigen- or mitogen-activated T cells (8) and was found to be essential for the generation of cytotoxic T cells (9). NK cells can also be maintained in active growth medium with IL-2 supplementation (1), and IL-2-supplemented medium has been used to clone NK cells (5). In addition, IL-2, like interferon, has been shown to augment NK cell activity (4, 13).

Recently an extraordinary outbreak of Kaposi's sarcoma and opportunistic infections including *Pneumocystis carinii* has been reported in homosexual men, intravenous drug abusers, Haitian immigrants, and hemophiliacs (23). Immunological studies revealed a severe defect in cell-mediated immunity. This acquired immune deficiency syndrome (AIDS) is characterized by lymphopenia, impaired T lymphocyte function, and reversal of the ratio of helper (OKT4) to suppressor (OKT8) cells. In addition, the patients with AIDS have reduced NK cell activity (7, 16, 20).

In this paper, we have examined the effect of IL-2 on NK cell activity in normal heterosexual men, healthy homosexual men, and homosexual men with AIDS.

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MATERIALS AND METHODS

Subjects. Peripheral blood was drawn in heparinized syringes from 8 heterosexual men with a mean of 33.8 ± 4.5 (standard deviation) years and ranging from 28 to 40 years, 5 healthy homosexual men with a mean age of 32.0 ± 6.9 years and ranging from 20 to 36 years, and 11 homosexual men with AIDS with a mean range of 36.8 ± 8.3 years and ranging from 28 to 57 years. The demographic data and clinical features of homosexual men with AIDS are shown in Table 1.

IL-2. Lectin-free TCGF (IL-2) was obtained from Associated Biomedic Systems, Inc., Buffalo, N.Y. It was prepared from pools of phytohemagglutinin-stimulated T lymphocytes recovered from up to 100 U of whole blood. IL-2 activity in these preparations was enhanced by the massive mixed lymphocyte reaction obtained from the pool. IL-2 in the media was partially purified by ammonium sulfate fractionation and DEAE-Sepharose chromatography (17). The IL-2 obtained was free of interferon, mitogenic activity for fresh lymphocytes, and inhibitors of DNA synthesis and long-term growth, but supported the growth of lymphocytes after activation by a mitogen or an antigen.

Target cells. The highly sensitive human myeloid leukemia cell line K562 was used. K562 cells were cultured in suspension in RPMI 1640 medium (GIBCO Laboratories, Grand Island, N.Y.) supplemented with 10% heat-inactivated fetal calf serum (GIBCO), penicillin-streptomycin (GIBCO), L-glutamine (GIBCO), and 10 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (GIBCO) and henceforth referred to as complete medium.

To label target cells with ^{51}Cr, 2×10^6 target cells suspended in 0.2 ml of complete medium were incubated with 100 μCi of $Na^{51}CrO_4$ (New England Nuclear Corp., Boston, Mass.) at 37°C in 5% CO_2 incubator for 1 h. After incubation, the target cells were washed three times in RPMI

TABLE 1. Demographic data, OKT4/OKT8 ratios, and NK cell activity in 11 homosexual men with AIDS

Patient no.	Infections or neoplasms	Age (yr)	Race[a]	Total lymphocytes per μl	OKT4/OKT8 ratio	% NK cell activity E/T ratio of 100:1 0% IL-2	% NK cell activity E/T ratio of 100:1 20% IL-2	% NK cell activity E/T ratio of 50:1 0% IL-2	% NK cell activity E/T ratio of 50:1 20% IL-2
1[b]	Kaposi's sarcoma	34	W	1,085	0.23	11.50	72.09	7.73	65.70
2[b]	Kaposi's sarcoma	28	W	630	0.03	14.96	67.01	3.38	65.66
3[b]	*Pneumocystis* pneumonia	57	H	594	0.55	17.40	41.01	9.30	29.58
4[b]	*Pneumocystis* pneumonia	32	W	1,748	0.26	2.00	43.48	1.19	27.82
5[b]	*Pneumocystis* pneumonia	40	W	527	0.29	16.78	64.84	7.18	58.70
6[b]	*Pneumocystis* pneumonia	44	W	1,023	0.50	7.98	28.07	3.78	20.92
7[b]	*Pneumocystis* pneumonia	30	B	245	0.63	7.43	48.09	4.75	32.04
8[c]	Candidiasis, mucosal lymphadenopathy	31	B		0.47	19.84	48.91	14.37	39.41
9[c]	Lymphadenopathy	33	B	2,108	0.61	16.08	61.93	9.42	51.13
10[d]	Pulmonary tuberculosis	40	B	1,496	0.44	11.13	28.36	11.55	23.31
11[d]	Mediastinal tuberculosis	36	H	816	0.30	2.00	38.64	1.41	21.01
Mean ± SD		36.8 ± 8.3		1,027 ± 593	0.4 ± 0.2	11.6 ± 6.1	49.3 ± 15.3	6.7 ± 4.2	39.6 ± 17.6

[a] H, Hispanic; W, white; B, black.
[b] AIDS as defined by the Centers for Disease Control, Atlanta Ga.
[c] AIDS prodrome.
[d] Presumed AIDS.

medium with 10% fetal calf serum, and viable cells were counted and suspended in complete medium.

Effector cells. Mononuclear effector cells were isolated from heparinized blood on Ficoll-Hypaque (Pharmacia Fine Chemicals, Piscataway, N.J.) gradients by density gradient centrifugation and washed three times with complete medium. These cells were cultured at 37°C in a 5% CO_2 humidified incubator in 17-mm polypropylene tubes with 5 ml of complete medium at a concentration of 10^6 cells per ml. Cells were cultured either in complete medium alone or with various concentrations of IL-2 in complete medium overnight. After incubation, the cells were washed in complete medium, the viable cells were counted, and the cell density was adjusted for the NK assay.

NK cell activity assay. Samples (100 μl each) of effector and ^{51}Cr-labeled target cell suspensions were added to flat-bottom microliter plates (Flow Laboratories, Inc., McLean, Va.) in triplicate and incubated at 37°C in a humidified 5% CO_2 incubator for 4 h. A 100-μl sample of the supernatant was removed, and the radioactivity was determined in a Beckman gamma spectrometer. The spontaneous release was determined for target cells incubated in medium alone (six wells), and maximum ^{51}Cr release was determined by lysing 100 μl of target cells with 0.1 ml 5% Triton X (six wells). The percent specific cytolysis was determined from counts per minute (cpm) with the following formula: percent cytotoxicity = [(cpm experimental release − cpm spontaneous release)/(cpm maximum release − cpm spontaneous release)] × 100. Spontaneous release ranged between 9.0 to 16.7% of the maximum release, with a mean of 11.6%.

OKT_4+ and OKT_8+ T cells. OKT_4+ and OKT_8+ cell proportions of circulating mononuclear cells were determined by using OKT4 and OKT8 antisera (Ortho Pharmaceutical Corp., Raritan, N.J.) by indirect immunofluorescence (14).

Statistical analysis. The statistical significance between the means was determined by independent Student's *t* tests and expressed as *P* values. The ratio of NK activity with IL-2 and without IL-2 was calculated from each individual's value.

RESULTS

NK cell activity of mononuclear cells from 11 homosexual men with AIDS, 5 healthy homosexual men, and 8 normal heterosexual men was tested against K562 myeloid tumor cells. The patients with AIDS as a group expressed a baseline mean NK activity of 11.6 ± 6.1 (standard deviation) with a range of 2 to 20% NK activity at a 100:1 effector/target (E/T) ratio (Table 1), whereas the normal heterosexual men exhibited a mean of 30.8 ± 11.0 cytotoxicity with a range of 18 to 53% NK activity at a 100:1 E/T ratio (Table 2). The difference between the two groups is significant ($P < 0.001$).

TABLE 2. Demographic data, OKT4/OKT8 ratios, and NK cell activity in eight normal heterosexual men

Control	Age (yr)	Race[a]	OKT4/OKT8 ratio	% NK cell activity E/T ratio of 100:1 0% IL-2	% NK cell activity E/T ratio of 100:1 20% IL-2	% NK cell activity E/T ratio of 50:1 0% IL-2	% NK cell activity E/T ratio of 50:1 20% IL-2
1	40	A	1.78	30.65	74.37	18.18	53.82
2	32	W	1.72	26.63	79.45	10.89	58.39
3	28	W	1.66	17.87	61.33	10.04	47.33
4	37	A	1.91	23.33	85.43	19.51	53.13
5	30	A	1.22	23.61	79.15	14.80	64.28
6	34	A	1.57	38.44	77.10	22.90	80.46
7	39	A	1.88	52.84	89.92	31.30	78.26
8	30	W	1.13	33.29	80.65	17.96	78.26
Mean ± SD	33.8 ± 4.5		1.6 ± 0.3	30.8 ± 11.0	78.4 ± 8.4	18.2 ± 6.8	64.2 ± 13.1

[a] Abbreviations as in Table 1. A, Asian.

The mean NK activity in healthy homosexual men was 16.8 ± 13.6 with a range of 4 to 34% NK activity at a 100:1 E/T ratio (Table 3), which is significantly decreased from that of normal heterosexual men ($P < 0.001$) (Table 4). The NK cell activity was reduced in three of the five healthy homosexual men. The OKT4/OKT8 ratio in the normal heterosexual group was 1.6 ± 0.3 compared with 1.0 ± 0.1 in healthy homosexual men and 0.4 ± 0.2 in homosexual men with AIDS.

Peripheral blood mononuclear cells (PBMC) from four normal heterosexual men were cultured either in complete medium alone or mixed with various concentrations of IL-2 at 37°C overnight and then assayed for cytolytic activity against K562 target cells. IL-2 was found to augment NK cell activity, which increased with concentration with a peak activity at 15 or 20% in the medium (Fig. 1).

PBMC from all subjects were then incubated either in complete medium or 20% IL-2 in complete medium overnight and assayed for NK cell activity against K562 targets. The NK cell activity in normal heterosexual men cultured in 20% IL-2 increased to 78.4 ± 8.4 from 30.8 ± 11.0 cytolytic activity at a 100:1 E/T ratio and from 18.2 ± 6.8 to 64.2 ± 13.1 at 50:1 E/T ratio (Table 4). The ratio of the increased NK activity was 2.7 ± 0.7 and 3.8 ± 1.0 at 100:1 and 50:1 E/T proportions, respectively, and was statistically significant ($P < 0.001$).

In healthy homosexual men, IL-2 incubation overnight increased the NK activity to 68.3 ± 17.6 from 16.8 ± 13.6 at a 100:1 E/T ratio and 54.0 ± 16.6 from 9.5 ± 8.0 at a 50:1 E/T ratio. This increase was 6.7 ± 4.2 times at a 100:1 E/T ratio and 8.8 ± 5.1 at a 50:1 E/T ratio (Table 4).

In homosexual men with AIDS, preincubation of effector cells overnight with 20% IL-2 increased the NK activity from 11.6 ± 6.1 to 49.3 ± 15.3 at a 100:1 E/T ratio and from 6.7 ± 4.2 to 39.6 ± 17.6 at a 50:1 E/T ratio. This increase of NK activity was 7.0 ± 6.9 and 9.1 ± 7.1 times at 100:1 and 50:1 E/T ratios, respectively, compared with values obtained without IL-2 in the medium. This increase is statistically significant ($P < 0.001$) (Table 4). The NK activity after IL-2 was clearly increased, but to absolute levels below that detected in the normal heterosexual and healthy homosexual subjects.

DISCUSSION

AIDS is characterized by a severe defect in cellular immunity with a reduction of the T lymphocyte helper subsets and decreased proliferative response to mitogens and antigens (20, 23). In addition, reduced NK cell activity was observed in homosexual men with this syndrome (7, 20). Siegal et al. (20) reported a herpes simplex virus-specific NK cell activity range of 0 to 15.7 lytic units per ml of blood in four homosexual men with AIDS compared with a range of 52 to 239 lytic units per ml of blood in controls. Similarly, Gerstoft et al. (7) observed a Molt-4 cell line-specific NK cell activity of 12, 27, and 54% of controls in three European homosexual men with AIDS. Recently, Lederman et al. (16) reported a K562-specific NK cell activity of 21.3 ± 3.8% in 11 hemophiliacs with AIDS who had been treated with lyophilized preparations of antihemophilic factor compared with 37.8 ± 2.8% in controls. Sears et al. (C. Sears, J. W. M. Gold, A. Ley, S. Cunningham-Rundles, B. Koziner, and D. Armstrong, Clin. Res. **30**:697A, 1982) studied healthy homosexual men at an upstate New York University campus, and they found that only 4 of 14 had normal NK cell activity. Only two of five of our healthy homosexual men had normal NK cell activity.

Dempsey et al. (4) cultured normal human PBMC either in complete medium alone or with TCGF at 37°C for various intervals before assaying for cytolytic activity against K562 targets. The NK cell activity of normal control PBMC incubated in complete medium increased initially with time and then decreased as the duration of the culture increased. In contrast, they observed that the NK cell activity of cells cultured in TCGF increased with time in culture and was maximal at 24 h. They found TCGF to augment NK cell activity in a manner directly proportional to concentration. Similarly, after overnight incubation of PBMC from four normal heterosexual men with various concentrations of lectin-free IL-2, we observed a dose-dependent augmentation of NK cell activity when compared with PBMC not incubated with IL-2. In addition the experiments reported here have shown preincubation of PBMC from normal heterosexual men, healthy homosexual men, and homosexual men with AIDS with IL-2 greatly enhanced the NK cytolytic activity.

Henny and co-workers showed previously that treatment of murine immune spleen cells with IL-2 was effective in potentiating in vitro NK cell activity (11, 15). This effect of IL-2 on NK cells was distinct from the known NK cell-boosting potential of interferon. The addition of both interferon and IL-2 to immune spleen cell cultures resulted in an additive augmentation of NK cytolytic activity over that obtainable by the addition of either lymphokine alone (15). Similar results were obtained with human lymphoid cells, suggesting that IL-2 enhancement of NK activity is independent from that of interferon (4). Minato et al. (18) analyzed the heterogeneity of NK cells in mice and identified at least four phenotypically distinct cytotoxic effector cells that differ not only in their surface markers, but also in their responsiveness to the regulatory influences of interferon and IL-2. Hefeneider et al. demonstrated that in vivo administration of IL-2 in mice augmented NK responses significantly (10).

The regulation of NK cell activity is complex and subject

TABLE 3. Demographic data, OKT4/OKT8 ratios, and NK cell activity in five healthy homosexual men

Patient no.	Age (yr)	Race[a]	Total lymphocytes per μl	OKT4/OKT8 ratio	% NK cell activity: E/T ratio of 100:1, 0% IL-2	% NK cell activity: E/T ratio of 100:1, 20% IL-2	% NK cell activity: E/T ratio of 50:1, 0% IL-2	% NK cell activity: E/T ratio of 50:1, 20% IL-2
1	36	B	1,350	0.93	3.63	38.90	2.32	25.40
2	20	W	1,242	1.02	34.42	74.27	20.74	64.64
3	32	W	3,002	1.13	8.22	86.10	4.55	66.17
4	36	W		0.83	9.50	72.35	4.75	55.30
5	36	B	3,055	1.00	28.01	69.72	15.20	58.66
Mean ± SD	32.0 ± 6.9		2,162 ± 1,001	1.0 ± 0.1	16.8 ± 13.6	68.3 ± 17.6	9.5 ± 8.0	54.0 ± 16.6

[a] Abbreviations as in Table 1.

TABLE 4. Effect of lectin-free human TCGF (IL-2) on NK cell activity in normal heterosexual men, healthy homosexual men, and homosexual men with AIDS

Expt. no.	Group	≃	Total no. of lymphocytes per μl[a]	OKT4/OKT8 ratio[a]	% IL-2	% NK cell activity[b]		NK activity ratio[c]	
						E/T ratio of 100:1[a]	E/T ratio of 50:1[a]	E/T ratio of 100:1	E/T ratio of 50:1
1	Normal heterosexual men	8	>1,500	1.6 ± 0.3	0	30.8 ± 11.0	18.2 ± 6.8	2.7 ± 0.7	3.8 ± 1.0
					20	78.4 ± 8.4	64.2 ± 13.1		
2	Healthy homosexual men	5	2,162 ± 1,001	1.0 ± 0.1	0	16.8 ± 13.6	9.5 ± 8.0	6.7 ± 4.2	8.8 ± 5.1
					20	68.3 ± 17.6	54.0 ± 16.6		
3	Homosexual men with AIDS	11	1,027 ± 593	0.4 ± 0.2	0	11.6 ± 6.1	6.7 ± 4.2	7.0 ± 6.9	9.1 ± 7.1
					20	49.3 ± 15.3	39.6 ± 17.6		

[a] Mean ± standard deviation.
[b] $P < 0.001$ in all cases.
[c] Ratio of NK cell activity with and without IL-2. The mean was calculated from each individual's value.

to modulation by various biological factors. The mechanism whereby IL-2 potentiates NK cytolytic activity is unknown. Interferon is known to increase both the efficacy of NK effector cells and their number by the recruitment of precursor cells (21). Similarly, IL-2 may function directly on NK cells or recruit pre-NK cells. Alternatively, IL-2 may potentiate NK cytolytic activity by functioning indirectly or in conjunction with another mediator like interferon. Recently, Riccardi et al. (19) used available information on the regulatory effects of interferon and IL-2 on the growth, differentiation, and activation of NK cells to formulate a model with positive and negative regulatory effects on the in vitro growth and differentiation of mouse NK cells.

The capacity of in vivo treatment of mice with IL-2 to boost NK responsiveness is suggestive evidence that in vivo IL-2 production and response may be of significance in regulating NK reactivity (10). This demonstrates the ability of purified IL-2 to function in vivo as an immune response modifier. IL-2-dependent T-cell proliferation technology had been applied to classic tumor therapy experimentation in animals to substantiate further the therapeutic effect of antitumor-reactive cytotoxic T-lymphocyte activity. Cheever et al. (2) reported the specific adoptive in vivo therapy of an established murine leukemia with syngenic cytotoxic T lymphocytes expanded in IL-2-dependent cultures. These studies in animals suggest that IL-2 may be of value as a therapeutic agent.

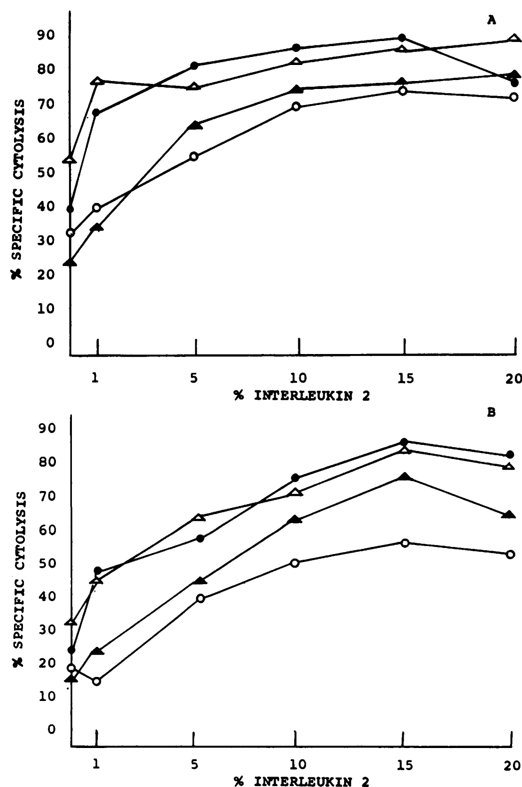

FIG. 1. Effects of preincubation of PBMC from four normal heterosexual men with various concentrations of lectin-free IL-2 overnight on NK cell activity with E/T ratios of 100:1 (A) and 50:1 (B).

Prince et al. (H. E. Prince, M. S. Gottlieb, V. Kermani-Arab, and L. J. Fahey, Fed. Proc. **42**:1334, 1983) reported that low proliferative response to phytohemagglutinin in AIDS patients may reflect defective IL-2 receptor appearance on stimulated cells. Recently, Ciobanu et al. (3) reported a homosexual patient with Kaposi's sarcoma with low endogenous IL-2 production in response to phytohemagglutinin. They also reported four other homosexual patients who had AIDS or Kaposi's sarcoma with an altered helper/suppressor ratio and a persistent defect in IL-2 production that was correctable in vitro by the addition of purified IL-2. There is an accumulating evidence to show that NK cells kill a variety of tumors (22). Since NK cells have an important role in immune surveillance (22), and IL-2 augments NK response, consideration of the use of IL-2 as a potential therapeutic agent to modify immune responses in disorders such as AIDS appears warranted.

ACKNOWLEDGMENTS

We thank C. Lopez and P. Fitzgerald for kindly providing K562 cell line, Elena Klein for testing interferon activity in IL-2, Mary Moriarty for technical assistance, and Yolanda Sandoval for preparation of the manuscript.

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