Partial Restoration of Impaired Interleukin-2 Production and Tac Antigen (Putative Interleukin-2 Receptor) Expression in Patients with Acquired Immune Deficiency Syndrome by Isoprinosine Treatment In Vitro

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Abstract

The in vitro effects of isoprinosine (ISO) on interleukin-2 (IL-2) production, the expression of Tac antigen (IL-2 receptor) on lymphocytes, and the ability of Leu 3(+) cells to absorb interleukin-1 (IL-1) were investigated in 10 patients with acquired immune deficiency syndrome (AIDS). In 9 of the 10 patients, production of IL-2 from mononuclear cells and Leu 3(+) cells was depressed; expression of Tac antigen on mononuclear cells and Leu 2(+) cells was found to be depressed in 9 of 10 patients. The ability of the Leu 3(+) lymphocytes to absorb IL-1 was depressed in all (four of four) patients studied. After ISO treatment, IL-2 production, Tac antigen expression and IL-1 absorption were restored to normal or near normal levels in most of the patients. These results suggest that ISO has an immunostimulating capacity in AIDS patients and that the potential of ISO in immune response restoration in AIDS patients deserves critical consideration.

Introduction

Acquired immune deficiency syndrome (AIDS)[1] is characterized by severe defects in immune responses, e.g., markedly depleted helper T cells (1, 2), impaired natural killer cell (NK) activity (3, 4), inverted helper/suppressor cell ratio (5, 6), depressed responses to mitogens and alloantigens (7), and depressed interleukin-2 (IL-2) production (8, 9). Although the etiology of AIDS is unknown, recent evidence suggests that retroviruses (HTLV-III) may be implicated as the primary cause of AIDS (10–13). It has been shown that IL-2 augments NK activity both in animals and in humans (14–16). Rook et al. (16) reported that IL-2 enhanced the depressed NK activity and cytomegalovirus-specific cytotoxic activity of lymphocytes from AIDS patients in vitro. Furthermore, NK activity and lymphocyte-proliferative activity were partly reconstituted when AIDS patients were treated with human recombinant IL-2 (9).

Isoprinosine (ISO) is an immune-potentiating drug demonstrated to augment NK activity in humans and animals (17–19). It can also enhance IL-2 production in mitogen-stimulated human lymphocytes in vitro (20). The rationale of IL-2 trials in AIDS patients at the National Institutes of Health (21) was based on the fact that IL-2 can augment both NK activity and cytotoxic T cell activity in these patients. We have demonstrated that ISO can augment human IL-2 production and NK activity in vitro. The present investigation reports the evaluation of the immunostimulating capacity of ISO in patients with AIDS in vitro in regard to IL-2 production and Tac antigen (putative IL-2 receptor) expression. The immune-restorative potential of ISO deserves critical consideration for application in patients with AIDS.

Methods

Patients and controls. Seven patients with characteristics of AIDS and three with symptoms of an AIDS-related complex were investigated. The criteria used to diagnose AIDS conform to those established by the Centers for Disease Control (22). AIDS-related complex symptoms were defined as those occurring without an opportunistic infection or Kaposi's sarcoma, but showing weight loss, fever, night sweats, fatigue, lymphadenopathy, and diarrhea; these occurred with an inverted ratio of T lymphocyte subsets (Table I). 30 normal adult heterosexual men were used as controls.

Drug. ISO (*N,N*-dimethylamino-2-propanol-*p*-acetamido-benzoate-inosine in a 3:1 molar ratio) was provided by Newport Pharmaceuticals International, Inc. (Newport Beach, CA). ISO was dissolved in RPMI-1640 medium and incubated with cells at an optimal concentration (100 μg per 10^6 cells/ml) (18).

Preparation of a supernatant containing IL-2. A modification of the method described by Mazingne et al. (23) was used to prepare supernatants that contained IL-2. 2×10^6 mononuclear cells/ml cultured in 2 ml of RPMI-1640 that contained ISO were incubated for 24 h at 37°C. Controls were incubated in medium alone. Cells were washed with RPMI-1640 and incubated for 24 h at 37°C in 2 ml of RPMI-1640 containing 5% fetal bovine serum and with a concentration 1 μg/ml of phytohemagglutinin (PHA). The supernatants were harvested and stored at −20°C until use.

Assay for IL-2 activity. For the IL-2 assay, 10^4 murine IL-2-dependent cells (CTL-20) were added to each well of a 96-well microtiter plate. Log_2 dilutions of the IL-2-containing supernatant were then added to the plate. After a 24-h incubation at 37°C with 5% CO_2, 1 μCi of [^{3}H]thymidine (5 Ci/mmol) was added to each well. The cells were incubated for an additional 4 h, and then harvested on glass filter strips. [^{3}H]thymidine was measured in a liquid scintillation counter and IL-2 concentrations in the samples were compared with the IL-2 standard.

Detection of Tac antigen. The method described by Tsudo et al. (24) was used for the detection of Tac antigen on mitogen-activated human lymphocytes. Anti-Tac antibody was used in an indirect

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1. *Abbreviations used in this paper:* AIDS, acquired immune deficiency syndrome; IL-1, interleukin-1; IL-2, interleukin-2; ISO, isoprinosine; NK, natural killer; PHA, phytohemagglutinin.

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Table I. Clinical Features and Lymphocyte Surface Markers in AIDS Patients

Patient	Sex	Diagnostic condition	Total T cell	Lymphocyte surface markers		
				Leu 3	Leu 2	Leu 3/Leu 2
			%	%	%	
1	M	Candidiasis Herpes simplex	47	14	45	0.31
2	M	Candidiasis KS	32	11	39	0.28
3	M	Candidiasis PCP	25	14	37	0.37
4	M	PCP Candidiasis	48	12	48	0.25
5	M	Candidiasis Herpes simplex	37	22	41	0.53
6	M	Herpes simplex	36	34	49	0.70
7	M	PCP Candidiasis	53	28	32	0.85
8	M	ARC	55	18	40	0.45
9	F	ARC	58	18	36	0.5
10	M	ARC	46	25	45	0.56
Normal controls (n = 30)			65.1±9.8	37.2±1.6	22.1±0.7	1.68

ARC, AIDS-related complex symptoms; F, female; KS, Kaposi's sarcoma; Leu 2, suppressor/cytotoxic cell, Leu 3, helper/inducer cell; M, male; PCP, *Pneumocystis carinii* pneumonia.

immunofluorescence method. Fluorescein isothiocyanate (FITC)-conjugated $F(ab')_2$ fraction of goat anti-mouse IgG antibody (Cappel Laboratories, Cochranville, PA) was employed as second antibody. The percentage of reactive cells was determined by counting at least 200 lymphocytes under a Zeiss ultraviolet microscope (Carl Zeiss, Inc., Thornwood, NY).

Lymphocyte subset studies. Mononuclear cells were separated by Ficoll-Hypaque gradients (25), and T cell subsets were determined by indirect immunofluorescence using monoclonal antibodies to Leu 2, Leu 3, and Leu 4 (Becton-Dickinson & Co., Mountain View, CA). Fluorescein isothiocyanate-conjugated goat anti-mouse IgG antibody as described previously was used as second antibody. The helper/suppressor ratio (Leu 3/Leu 2) was calculated as the number of peripheral blood lymphocytes stained with anti-Leu 3 divided by the number of lymphocytes stained with anti-Leu 2.

T cell subset isolation. T lymphocytes were separated by using nonadherent mononuclear cells resettled with 2-aminoethylisothiouronium bromide hydrobromide-treated sheep erythrocytes and then followed by lysis of the attached erythrocytes with 0.85% Tris-buffered NH_4Cl solution.

Leu 2(+) and Leu 3(+) lymphocytes were isolated by the elimination method described by Timonen et al. (25). In brief, 0.2 ml of a 1:10 dilution of anti-Leu 2 or anti-Leu 3 antibodies was added to 5×10^6 lymphocytes in 0.1 ml of medium. The mixture was kept on ice for 30 min before addition of 1 ml of a 1:10 dilution of guinea pig complement. After further incubation for 60 min at 37°C, the cells were washed and resuspended at 1×10^6 cells/ml in RPMI-1640 containing 5% fetal bovine serum. The efficiency of the procedure in removing cells was checked by staining the remaining lymphocytes with anti-Leu 2 and anti-Leu 3 antibodies.

Interleukin-1 (IL-1) activity assay. IL-1 was prepared by stimulation of normal human monocytes by the method similar to Gery et al. (26). Monocytes (10^6 cell/ml) were cultured with lipopolysaccharide (5 μg/ml) for 48 h at 37°C and then dialyzed for 24 h against RPMI-1640 medium. For absorption studies, 10^6 Leu 3(+) cells were added to IL-1 preparations in the presence or absence of PHA and incubated for 24 h at 37°C. The supernatants were recovered by centrifugation and partially purified by Sephadex G-100 column chromatography to remove the PHA in the supernatant and then concentrated to the original volume. IL-1 activity was assayed by the method described by Simons et al. (27, 28). In brief, EL-4 cells (thymoma cells) were cultured for 24 h with calcium ionophore A23187 (Sigma Chemical Co., St. Louis, MO) (2.5×10^{-7} M) and the partially purified supernatant. The IL-2 activity in 24-h culture supernatants was assayed by using the CTL-20 cell line as described above. Duplicate plates were prepared containing the partially purified IL-1 supernatant and A23187 but without EL-4 cells to measure the endogenous IL-2 activity.

Statistical analysis. Statistical comparisons were made by means of the two-tailed t test.

Results

Clinical features and lymphocyte enumeration. The clinical data and lymphocyte enumeration concerning the 10 patients are summarized in Table I. Patients 1–7 met the strict criteria of the Centers for Disease Control for a diagnosis of AIDS (22); the other three had no demonstrable opportunistic infection and were thus classified as having AIDS-related complex symptoms. All patients had decreased numbers of Leu 3(+) T

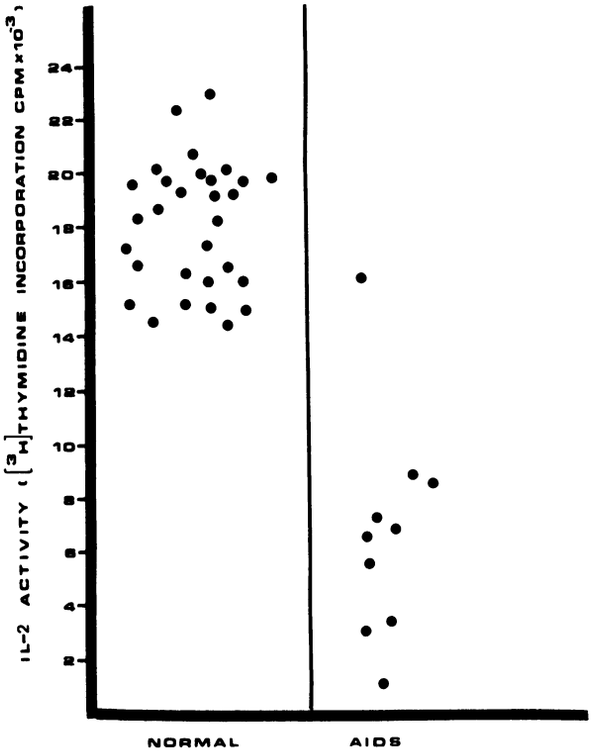

Figure 1. Production of IL-2 in patients with AIDS and normal controls.

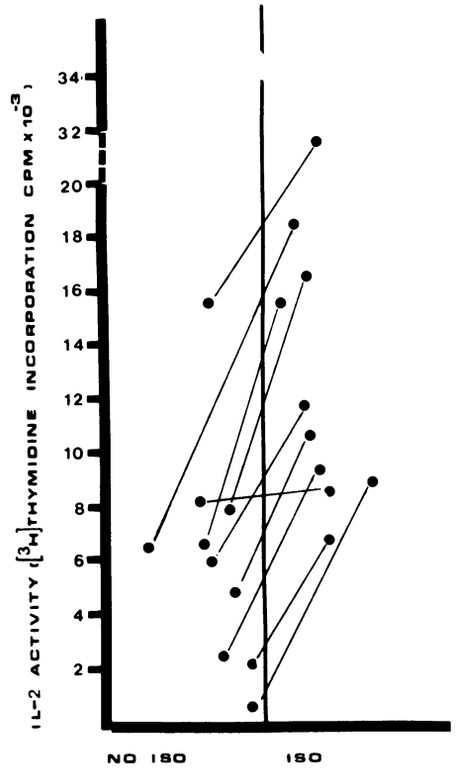

Figure 3. In vitro effects of ISO on mononuclear cell IL-2 production in AIDS patients.

cells. The ratio of helper/suppressor (Leu 3/Leu 2) was low in all patients.

Effects of isoprinosine on IL-2 production. Fig. 1 shows the production of IL-2 by PHA-stimulated mononuclear cells in 30 normal controls and 10 AIDS patients; all AIDS patients demonstrated decreased IL-2 production except patient 7. The IL-2 production in all 30 controls was normal. Treatment of mononuclear cells with ISO increased the production of IL-2 in all normal controls and 9 of 10 patients with AIDS (Figs. 2 and 3). Patient 6 was the only patient who failed to show augmentation in IL-2 production after ISO treatment. Table II shows the production of IL-2 by Leu 3(+) T cells in patients

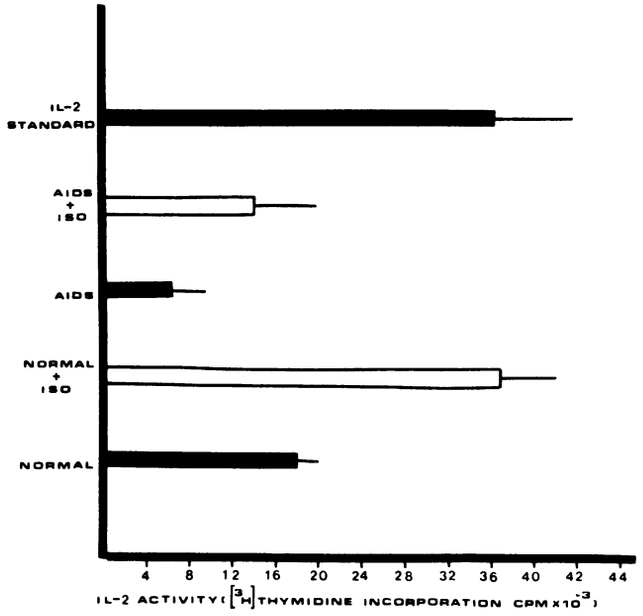

Figure 2. In vitro effects of ISO on IL-2 production in AIDS patients and normal controls. Results are expressed as mean±SE (n = 10 for AIDS patients; n = 30 for normal controls).

Table II. Effect of ISO In Vitro on IL-2 Production by Leu 3(+) T Cells

Patient	IL-2 production	
	Without ISO	With ISO
	cpm	*cpm*
1	2,971±542	28,312±2,162*
2	5,071±434	10,723±974*
3	4,927±506	8,745±679*
4	1,124±293	7,264±341*
5	2,014±367	8,726±429*
6	7,924±546	7,501±612
7	14,102±1,243	28,312±2,162*
8	5,291±419	10,942±1,984*
9	7,192±619	12,420±820*
10	6,982±782	17,243±2,141*
Normal (n = 20)	21,243±3,145.6	34,265±6,014.2*

IL-2 activity was tested in a microassay using the IL-2-dependent cell line CTL-20. Cell proliferation was measured by [^{3}H]thymidine uptake during incubation for 24 h at 37°C.
* Statistical significance, $P < 0.01$ (t test).

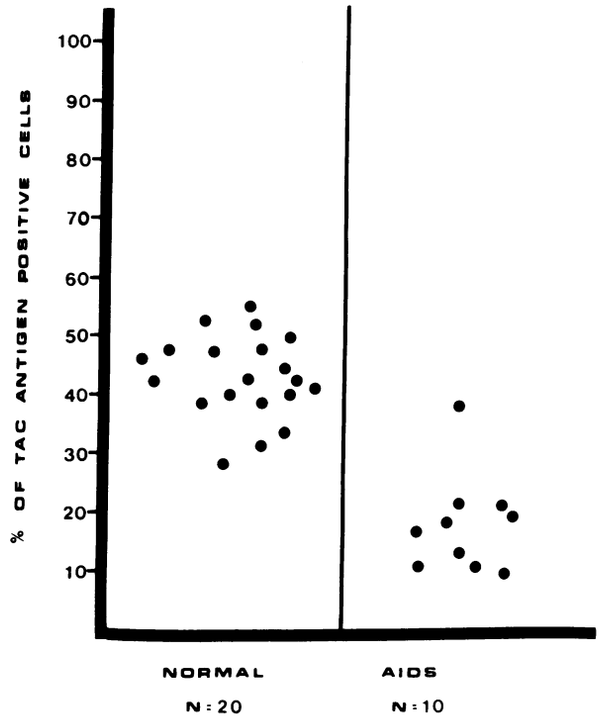

Figure 4. Tac antigen expression on mononuclear cells in AIDS patients and normal controls (n = 10 for AIDS patients; n = 20 for normal controls).

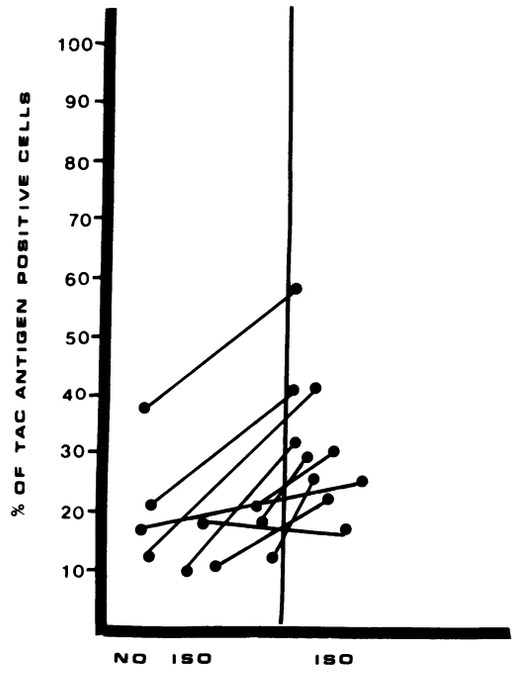

Figure 5. In vitro effects of ISO on Tac antigen expression in AIDS patients.

and normal controls as well as the effect of ISO on IL-2 production of Leu 3(+) T cells. IL-2 production was significantly increased after treatment with ISO in both normals and patients (except patient 6).

ISO effects on Tac antigen expression. Fig. 4 shows the Tac antigen expression on mononuclear cells after PHA stimulation in AIDS patients and normal controls. Mononuclear cells were treated with PHA (1 μg/10^6 cells per ml) for 24 h at 37°C before the determination of Tac antigen-positive cells by the immunofluorescence assay. All AIDS patients had decreased numbers of Tac-positive cells except patient 7. Normal controls showed normal expressions of Tac antigen. ISO increased the percentage of Tac(+) cells in both patients and normal controls (Table III). Fig. 5 shows the effect of ISO on the expression of Tac antigen in AIDS patients. Significant increases in the percentage of Tac(+) cells were observed in nine patients with AIDS with the exception of patient 6. Table IV shows the effects of ISO on Tac antigen expression on Leu 2(+) T lymphocytes in AIDS patients.

Effect of ISO on IL-1 absorption. Table V shows the effect of ISO on IL-1 absorption by Leu 3(+) T lymphocytes in four

Table III. Expression of Tac Antigen After Mitogen Stimulation of Lymphocytes in Normal Humans and AIDS Patients

	Percentage of Tac antigen-positive cells	
Lymphocyte source	Without ISO	With ISO
	%	%
Normal humans (n = 20)	43±6.7	59±5.6*
AIDS patients (n = 10)	18±8.1	31±11.8*

PHA was used at 1 μg/ml and incubated for 24 h at 37°C. Percentage of Tac antigen-positive cells was determined by immunofluorescence.

* Statistical significance, $P < 0.01$ (t test).

Table IV. Effect of Isoprinosine In Vitro on Tac Antigen Expression After Mitogen Stimulation of Leu 2(+) T cells in AIDS Patients

	Percentage of Tac Antigen-positive cells	
Patient	Without ISO	With ISO
	%	%
1	11	37*
2	15	28*
3	10	21*
4	9	19*
5	18	28*
6	14	12
7	31	51*
8	20	58*
9	15	27*
10	13	26*
Mean±SE	15.6±6.3	28.7±11.0*

Percentage of Tac antigen-positive cells was determined by immunofluorescent assays using anti-Tac antibody. PHA used at 1 μg/ml per 10^6 cells and incubated for 24 h at 37°C. Isoprinosine was used at 100 μg/ml per 10^6 cells.

* Statistical significance, $P < 0.01$ (t test).

Table V. Effect of Isoprinosine In Vitro on IL-1 Uptake by Leu 3(+) T Cells in Normal Humans and AIDS Patients

Patients		IL-1 activity left after absorption*		Percentage of enhancement
		Without ISO	With ISO	
		cpm	*cpm*	%
1	A	12,094	10,299	—
	B	7,342	3,071	58.1
2	A	10,943	11,042	—
	B	8,972	5,436	39.4
5	A	12,194	10,139	—
	B	6,324	4,126	34.7
6	A	9,890	10,434	—
	B	7,269	4,301	40.8
Normal	A	11,426	10,123	—
	B	2,641	2,076	21.3
Normal	A	9,342	10,434	—
	B	3,145	2,014	35.9
Normal	A	10,487	11,409	—
	B	2,592	1,973	23.8
Normal	A	11,352	12,104	—
	B	3,612	2,012	44.2
Medium alone		12,512	12,730	—

IL-1 was prepared by stimulation of normal human monocytes (10^6 cells/ml) with lipopolysaccharide (5 μg/ml) for 48 h at 37°C and dialyzed for 24 h against RPMI-1640 medium. 10^6 Leu 3(+) T cells were added to IL-1 preparations in the presence (B) or absence (A) of PHA and incubated for 24 h at 37°C. The supernatants were recovered by centrifugation and then assayed for IL-1 activity. ISO was used at a concentration of 100 μg/ml per 10^6 cells. Duplicate plates were prepared containing IL-1 supernatant, A23187 but without EL-4 cells to measure the endogenous IL-2 activity.
* Total counts per minute − endogenous counts per minute.

AIDS patients and four normal controls. Augmentation of IL-1 absorption was observed as a result of ISO treatment after PHA stimulation of all patients and normal controls tested.

Discussion

ISO effects on IL-2 production and Tac antigen expression in 10 AIDS patients and normal controls were investigated in vitro. Of 10 patients, 9 had depressed IL-2 production and decreased expression of Tac antigen after mitogen stimulation. Similar findings have been reported by other investigators (8, 9). However, we have shown that significant augmentation in IL-2 production occurred in 9 of 10 patients with AIDS when their mononuclear cells were treated with ISO in vitro. Augmentation of IL-2 production was also demonstrated when normal control mononuclear cells were treated with ISO. It is well documented that one striking feature of the immune system of AIDS patients is lymphopenia, predominantly due to a selective defect in the helper/inducer T lymphocyte subset (Leu 3 or OKT4). It has been suggested that the Leu 3 or OKT4 cell is both the main producer of IL-2 and also the responder to IL-1 (29–31). For this reason, we investigated the effect of ISO on IL-2 production by Leu 3(+) cells of AIDS patients. The data clearly show that IL-2 production by Leu 3(+) cells from AIDS patients is depressed, and that in vitro ISO treatment of the Leu 3(+) cells augments IL-2 production significantly. Our results also show that the ability of Leu 3(+) cells of AIDS patients to absorb IL-1 is suppressed as compared to normal controls and that treatment of the Leu 3(+) cells with ISO augments IL-1 absorption. Inasmuch as the supernatant was subjected to partial purification by Sephadex G-100 column chromatography for the removal of PHA, we are certain that the IL-2 activity produced by EL-4 cells in the IL-1 assay was induced by IL-1 and A23187, and not by PHA and A23187. In most cases, IL-1 is required for the production of IL-2, and defects in IL-1 absorption may be one reason for the depressed IL-2 production in AIDS patients. ISO augmented the expression of Tac antigen of mononuclear cells in AIDS patients after PHA stimulation. Anti-Tac antibody is reactive with activated and functionally mature human T lymphocytes including concanavalin A-induced suppressor cells, cytotoxic cells, and PWM-stimulated helper and suppressor cells (23, 32). T cells cultured with concanavalin A or allogeneic cells, but not reactive with anti-Tac antibody, had little or no suppressor or killer cell activity. Anti-Tac antibody is not reactive with resting T cells. It has been suggested that Leu 2(+) or OKT8(+) lymphocytes respond best to IL-2. In this regard, we investigated the Tac antigen expression of Leu 2(+) cells after PHA stimulation. Our data show that the expression of Tac antigen on Leu 2(+) cells was depressed in AIDS patients and that ISO augmented the Tac antigen expression to normal or near normal levels. Depressed Tac antigen expression may directly or indirectly influence the utilization of IL-2 in vivo in AIDS patients. NK activity augmentation by treatment with IL-2 in vitro and in vivo has been reported by many investigators (14–16, 30) and ISO has been found to increase various immune responses both in vitro and in vivo (18–20). Thus, ISO can be used in a variety of disorders to improve host resistance. Other examples include interferon production (33), influenza infection (34), and herpes (35), as well as human rhinovirus infections (36). We herein demonstrate that ISO can augment IL-2 production and expression of Tac antigen as well as absorption of IL-1 by Leu 3(+) cells in AIDS patients. All of these reactivities are undoubtedly interrelated in the stimulation of the immune response. Increased expression of Tac antigen may increase the utilization of IL-2 and indirectly increase NK activity. The reason for the lack of expression of the Tac antigen (and conceivably of the IL-2 receptors) is unknown, but may be due to blockage or binding of the receptor by a retrovirus, as is true of other viruses for other receptors (e.g., HSV-1 virus to dopamine receptor (37). This possibility is currently under exploration. Mühlradt and Opitz (38) reported that IL-2 clearance from the blood of normal mice was rapid with a 3-min half-life.

Thus, we speculate that the utilization of immune potentiators that can augment IL-2 production in vivo may be more beneficial than IL-2 treatment per se. Because the mechanism of ISO action appears to be stimulation of a dysregulated immune system and because of the depressed state of the immune system in AIDS patients, the in vivo use of ISO in AIDS patients may be beneficial.

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