JCI The Journal of Clinical Investigation

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J Clin Invest. 1996;98(3):616-621. https://doi.org/10.1172/JCI118831.

Research Article

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Activation of Antigen-induced Lymphocyte Proliferation by Interleukin-15 without the Mitogenic Effect of Interleukin-2 that may Induce Human Immunodeficiency Virus–1 Expression

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Abstract

The newly identified cytokine, IL-15 enhanced antigen-induced proliferation of PBMC obtained from HIV-1–seropositive subjects. When compared to IL-2 which enhanced both spontaneous and antigen-induced lymphocyte proliferative responses, IL-15 rarely increased spontaneous lymphocyte proliferation. Additionally, in cultures of lymphocytes obtained from 15 HIV-1–infected patients with < 300 circulating $CD4^+$ lymphocytes/μl IL-15 induced significant HIV-1 expression (46, 21, and 71 pg/ml) in only 3 of 15 experiments and IL-2 induced significant HIV-1 expression (range 16–> 5000 pg/ml) in 11 of 15 experiments ($P < 0.01$, Fischer's exact test). Simultaneous assays of cytokine-induced spontaneous lymphocyte proliferation and HIV-1 expression revealed similar dose–response relationships for induction of HIV-1 and lymphocyte proliferation by IL-2. Thus, IL-15 helps to correct the impaired proliferative response of $CD4^+$ lymphocytes from HIV-1–infected persons without the mitogenic effect of IL-2 that also may induce HIV-1 expression. (*J. Clin. Invest.* 1996. 98:616–621.) Key words: cellular proliferation • cytokines • HIV • human • T lymphocytes

Introduction

The recently identified cytokine IL-15 shares many biological properties with IL-2 (1). IL-15 binds the β and γ subunits of the IL-2 receptor and like IL-2, enhances proliferation of phytohemagglutinin-activated peripheral blood T cells and induces the generation of cytolytic effector cells (1). On the other hand the IL-3–dependent cell line 32D, which expresses IL-2Rα, β, and γ and proliferates in response to IL-2, responded poorly to stimulation with IL-15. Thus IL-15 has biological activities distinguishable from those of IL-2 (1).

HIV-1 infection is characterized by profound abnormalities of T cell numbers and function. T cell proliferation is impaired (2–5) and this is associated with diminished production of IL-2 in response to activation (6). Exogenous IL-2 may partially correct some impaired proliferative responses of lymphocytes obtained from HIV-1–infected persons (7–9). Administration of IL-2 to HIV-1–infected patients increases the numbers of circulating $CD4^+$ lymphocytes (10). This may not be without cost as IL-2 activates HIV-1 expression in vitro (11) and transiently increases plasma HIV-1 levels after in vivo administration (10). We compared the effects of IL-2 and IL-15 on spontaneous and antigen-induced proliferation of lymphocytes obtained from HIV-1–seropositive subjects. We found that in contrast to IL-2, equimolar concentrations of IL-15 specifically enhanced antigen-induced lymphocyte proliferation without affecting spontaneous proliferation. Moreover in contrast to IL-2, IL-15 rarely activated HIV-1 expression.

Methods

Cell preparation and culture conditions. PBMC were prepared from heparinized venous blood by Ficoll-hypaque (Pharmacia LKB Biotechnology Inc., Uppsala, Sweden) density sedimentation. Cells were maintained in complete medium, consisting of RPMI-1640 (Biowhittaker, Walkersville, MD) supplemented with 10% heat-inactivated pooled human serum (Gemini Bioproducts, Calabasas, CA), 2 mM L-glutamine, 10 mM Hepes buffer, 100 U/ml penicillin, 100 μg/ml streptomycin at 37°C in a humidified 5% CO_2-enriched incubator.

Lymphocyte proliferation assay. One hundred thousand PBMC were cultured in complete medium with candida antigen (CASTA;[1] Greer Laboratories, Lenoir, NC, 50 μg/ml), streptokinase (Boehringer Mannheim, Mannheim, Germany, 5 μg/ml), IL-15 (recombinant simian IL-15 Lot 4299-98, Immunex Corp., Seattle, WA), or IL-2 (recombinant human IL-2, Chiron, Emeryville, CA) as indicated. ^{3}[H]Thymidine incorporation was measured after 7 d of culture using scintillation counting. The activity of the IL-15 in a CTLL cell line proliferation assay (1) was 2.9×10^5 U/μg whereas the activity of the IL-2 was $7–8 \times 10^5$ U/μg.

Measurement of HIV-1 production. One million PBMC obtained from HIV-1–infected donors were suspended in triplicate 1-ml wells to which was added PHA (Sigma Chemical Co., St. Louis, MO), IL-

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Received for publication 27 November 1995 and accepted in revised form 20 May 1996.

J. Clin. Invest.

0021-9738/96/08/0616/06 $2.00
Volume 98, Number 3, August 1996, 616–621

1. *Abbreviation used in this paper:* CASTA, Candida albicans skin test antigen.

15, or IL-2. Supernatants were harvested after 14 d of culture and HIV-1 core protein was measured by ELISA (Coulter Diagnostics, Hialeah, FL). The lower limit of detection of this assay was 8 pg/ml as defined by linearity of the standard curve to this level.

Results

Effects of IL-15 and IL-2 on spontaneous and antigen-induced proliferation of PBMC from HIV-1–seropositive subjects. Shown in Fig. 1 *A* and *B* are results of a representative experiment using lymphocytes obtained from one HIV-1 seropositive subject. Addition of IL-2 or IL-15 to PBMC cultures containing CASTA enhanced lymphocyte proliferation. Spontaneous proliferation was increased in the presence of IL-2 but not IL-15. In two experiments IL-2 and IL-15 also enhanced streptokinase-induced lymphocyte proliferation (not shown).

Shown in Table I are the effects of IL-2 and IL-15 on spontaneous and CASTA-induced lymphocyte proliferative responses using PBMC from 22 HIV-1–seropositive subjects. IL-15 rarely affected spontaneous lymphocyte proliferation with cytokine induced proliferation never exceeding baseline in seven experiments (negative Δcpm) and a median peak enhancement of only 241 cpm. In fact, slopes ($\Delta cpm/\Delta \log_{10}$ IL-15 concentration) were flat (as shown in Fig. 1 *A*) in all but 1 of 22 experiments. As compared to the IL-15 effect, IL-2 enhanced spontaneous lymphocyte proliferation in 21 of 22 experiments with a median peak enhancement of 25,233 cpm ($P < 0.01$, Wilcoxon-Rank Sum test).

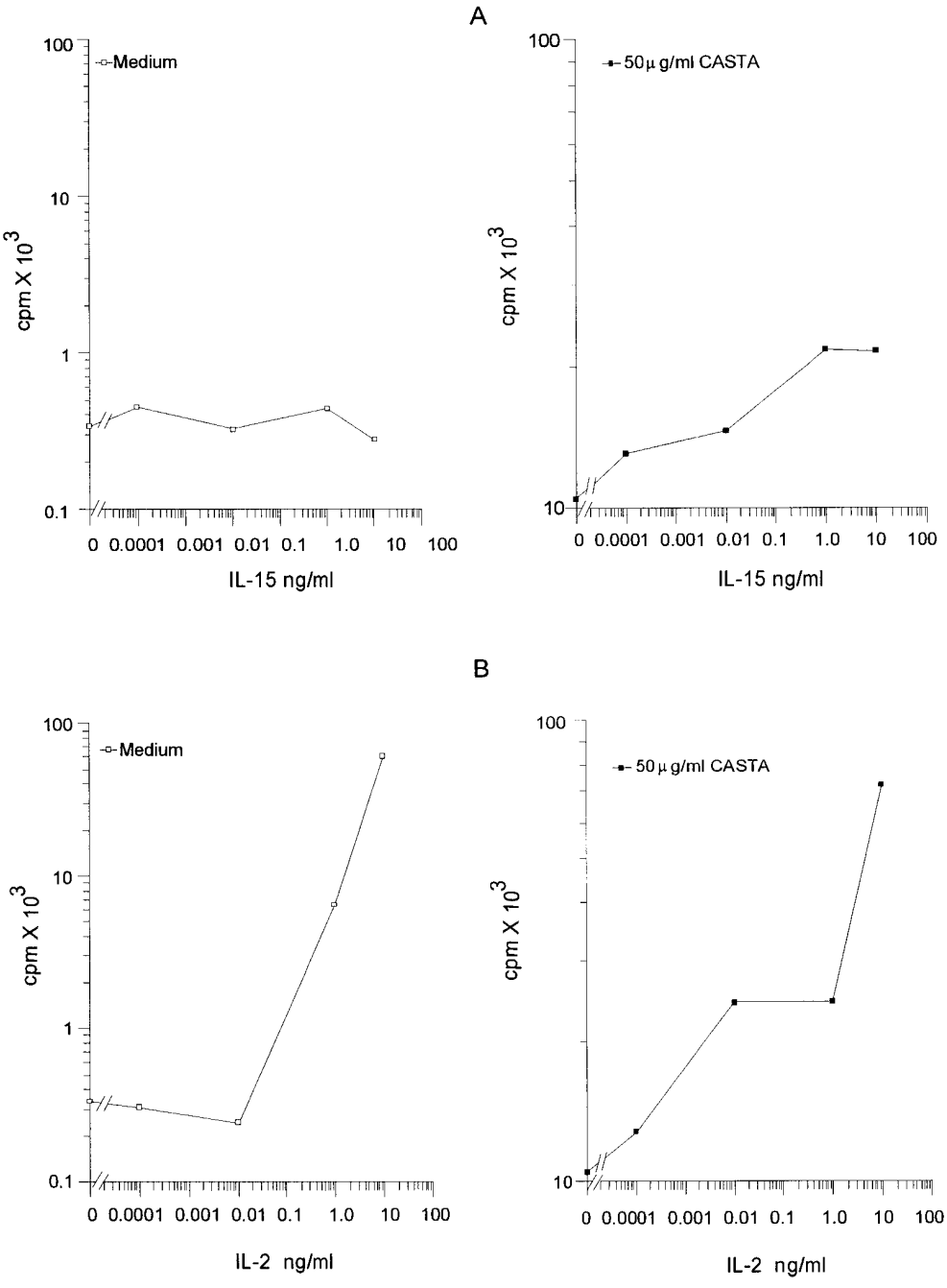

Figure 1. Cytokine effects on spontaneous and CASTA-induced lymphocyte proliferation. PBMC prepared from one HIV-1–infected subject were cultivated for 7 d with or without 50 μg/ml CASTA in the absence or presence of varying concentrations of IL-15 (*A*) or IL-2 (*B*). [^{3}H]Thymidine incorporation is expressed in counts per minute.

Table I. Cytokine Effects on Lymphocyte Proliferation

Patient (*n*)	Age (*y*)	Sex	CD4 (cells/μl)	Antiviral	cpm × 10^3		Peak Δcpm × 10^3 (fold enhancement)			
					Medium	Medium + CASTA	IL-2	IL-15	IL-2 + CASTA	IL-15 + CASTA
1	45	M	0	AZT	0.7	0.5	10.7(15)	−0.1(0)	8.4(16)	0.4(1.8)
2	34	M	0	AZT, ddI	0.5	0.4	18.3(41)	0.2(1.5)	19.8(58)	0.4(2.2)
3	47	M	20	AZT, ddI	0.9	6.1	1.9(3.2)	−0.02(0)	22.8(4.7)	12.9(3.1)
4	43	M	280	unknown	0.2	9.3	47.7(202)	1.2(5.8)	31.3(4.3)	21.6(3.3)
5	38	F	280	AZT	0.3	9.1	34.7(119)	0.3(1.9)	32.8(4.6)	12.4(2.4)
6	27	M	310	AZT	1.9	25.7	2.2(2.1)	0.7(1.3)	31.9(2.2)	18.8(1.7)
7	40	M	320	AZT	0.4	3.4	5.4(14)	0.2(1.5)	25.9(8.7)	4.5(2.3)
8	35	M	330	AZT	6.8	1.9	−0.3(0)	−0.3(0)	4.9(3.5)	−0.6(0)
9	30	F	360	unknown	0.4	13.7	25.7(62)	−0.05(0)	17.2(2.3)	20.7(2.5)
10	32	M	360	AZT, ddc	0.5	0.5	53.9(100)	3.4(7.2)	74.7(150)	69.2(139)
11	41	M	386	ddI	0.3	17.9	57.4(201)	17.8(63)	19.4(2.1)	12.6(1.7)
12	34	F	400	AZT	4.4	15.5	2.6(1.6)	3.5(1.7)	28.7(2.8)	25.2(2.6)
13	26	M	560	unknown	0.3	13.9	77.0(259)	0.2(1.3)	37.0(3.7)	1.9(1.1)
14	32	M	570	AZT	0.3	10.4	60.3(182)	0.1(1.3)	61.6(6.8)	11.4(2.1)
15	26	F	580	AZT	0.2	13.9	49.1(94)	0.4(1.7)	45.5(3)	23.3(2)
16	32	M	590	none	0.3	17.9	24.8(80)	0.2(0.8)	36.9(3)	21.6(2.2)
17	29	M	600	none	2.3	28.1	4.8(3)	−0.8(0)	31.6(2.1)	13.2(1.5)
18	39	M	620	AZT	1.4	125.4	10.6(8.7)	1.0(1.7)	10.6(1.1)	22.9(1.2)
19	47	M	630	none	1.3	41.4	37.9(30)	−0.1(0)	55.4(2.3)	14.5(1.4)
20	30	M	780	AZT	0.9	91.4	85.3(87)	1.3(2.3)	39.0(1.4)	47.2(1.5)
21	35	M	840	none	1.4	23.6	48.9(48)	0.6(1.5)	47.9(1.6)	62.3(1.8)
22	34	M	900	none	1.0	75.8	18.8(14)	−0.03(0)	47.8(3)	36.8(2.6)

Cytokine effects on spontaneous and CASTA-induced lymphocyte proliferation. PBMC were cultivated in quadruplicate or sextuplicate microtiter wells in the presence or absence of CASTA to which medium or graded concentrations ($10^{-4} - 10^{1}$ ng/ml) of IL-2 or IL-15 was added. ^{3}[H]Thymidine incorpoation was measured after 7 d culture. Peak Δcpm for spontaneous proliferation = maximum proliferation with cytokine − proliferation in medium without cytokine. Peak Δcpm for CASTA responses = maximum proliferation in presence of CASTA + cytokine − proliferation in presence of CASTA alone. Fold enhancement for spontaneous proliferation = peak proliferation in the presence of cytokine/proliferation in the absence of cytokine. Fold enhancement for CASTA responses = peak proliferation in the presence of CASTA + cytokine/proliferation in the presence of CASTA alone.

Both IL-2 and IL-15 enhanced CASTA-induced proliferation and for both IL-2 and IL-15 (though most apparent for IL-15), the magnitude of enhancement as indicated by Δcpm tended to be greater among patients with higher $CD4^+$ lymphocyte counts. At these peaks however, much of the proliferation induced by IL-2 in the presence of antigen was also seen in the absence of antigen. In fact, the peak enhancement (Δcpm) induced by IL-2 in the absence of antigen exceeded the peak antigen-driven enhancement in 9 of 22 experiments and approximated antigen-driven enhancement in 5 of the remaining experiments. In contrast, peak enhancement (Δcpm) for spontaneous proliferation in the presence of IL-15 exceeded the peak enhancement for antigen-induced responses in only 1 of 22 experiments ($P < 0.01$, Fischer's exact test).

To ascertain if the failure of IL-15 to induce spontaneous lymphocyte proliferation was related to differences in the dose–response curve for these cytokines, additional experiments were performed using higher concentrations of both IL-2 and IL-15 and PBMC from six HIV-1–seropositive subjects with CD4 counts ranging from 20 to 610/μl (Table II). In these experiments, IL-2 enhanced both antigen-dependent and antigen-independent lymphocyte proliferation as before. IL-15 also significantly increased CASTA-induced proliferation in PBMC from each subject ($P < 0.05$, Student's *t* test). Using concentrations of IL-15 from 0.0001 to 200 ng/ml, we observed no enhancement of spontaneous lymphocyte proliferation by this cytokine in any subject.

Cytokine-induced activation of HIV-1 expression. To ascertain if the mitogenic effect of IL-2 might enhance spontaneous expression of HIV-1 and to ascertain if IL-15 also could induce HIV-1 expression from latently infected cells, PBMC obtained from 21 HIV-1–infected patients were incubated for 2 wk in culture medium or medium supplemented with graded concentrations of recombinant cytokine and supernatant p-24 antigen levels were measured. Cultures prepared from 12 of 21 patients spontaneously expressed HIV-1 p24 antigen (> 8 pg/ml) after 2 wk cultivation in the absence of exogenous cytokines (Table III). In most instances, the level of viral production was low (in all but one instance, < 200 pg/ml). This level of viral production was enhanced in 13 of 21 experiments when PHA was added. Addition of IL-15 significantly increased (two- to fivefold) supernatant levels of HIV-1 p24 antigen in 4 of 21 experiments. In contrast, significantly enhanced HIV-1 production (1.6 to > 300-fold) was observed in the supernatants of 12 of 21 cultures in the presence of IL-2 ($P < 0.03$, Fischer's exact test). Enhancement of HIV-1 production by IL-2 was more likely in experiments where HIV-1 expression was enhanced by the T cell mitogen PHA ($P < 0.04$, Fischer's exact test).

Induction of HIV-1 expression by IL-2 was related to the circulating $CD4^+$ lymphocyte count. Cultures from only one of six

Table II. High Dose Cytokine Effects on Lymphocyte Proliferation

Patient (*n*)	Age (*y*)	Sex	CD4 (cells/μl)	Antiviral	cpm × 10^3		Peak Δcpm × 10^3 (fold enhancement)			
					Medium	Medium + CASTA	IL-2	IL-15	IL-2 + CASTA	IL-15 + CASTA
33	35	M	20	AZT, ddI	1.5	1.2	20.2(14)	0.4(1.4)	11.9(10)	2.4(2.9)
34	30	F	54	ddI	1.1	1.0	14.6(127)	0.5(1.4)	13.1(123)	1.2(2.1)
35	33	M	200	AZT, ddI	6.7	13.8	98.3(16)	0.05(1.1)	60.3(5.3)	14.6(2.1)
36	42	M	250	AZT	0.6	19.1	217.7(332)	0.4(1.6)	64.6(3.8)	6(1.3)
37	44	M	300	AZT	0.5	13.3	24.5(44)	0.3(0.9)	80.4(7.1)	16.5(2.3)
38	37	F	610	AZT	3.6	83.1	100.8(29)	2.8(1.7)	18.6(1.2)	34.2(1.4)

Legend same as in Table I, except that concentrations of IL-2 and IL-15 ranged $10^{-4}-2\times10^2$ ng/ml.

patients with $CD4^+$ cell counts exceeding 300/μl showed enhanced p-24 expression induced by IL-2 while cultures from 11 of 15 patients with $CD4^+$ counts < 300/μl showed significantly enhanced p-24 expression in the presence of IL-2 ($P < 0.05$, Fischer's exact test). In performing these 15 experiments using lymphocytes obtained from patients with < 300 $CD4^+$ lymphocytes/μl, IL-2 enhanced HIV-1 expression in 11 whereas IL-15 enhanced HIV-1 production in only 3 ($P < 0.01$, Fischer's exact test).

For the experiments shown in Table III *B*, assays of lymphocyte proliferation also were performed to concurrently examine the effects of a broad range of IL-2 and IL-15 concentrations on lymphocyte proliferation and HIV-1 expression. In three experiments, HIV-1 expression also was induced and the data from these experiments are shown in Fig. 2. In performing each of these experiments using PBMC from patients with CD4 counts < 300/μl, both IL-2 and IL-15 enhanced the lymphocyte proliferative responses to CASTA and IL-2 enhanced spontaneous lymphocyte proliferation. In each of these experiments the induction of HIV-1 expression by IL-2 reflected closely the mitogenic effect of this cytokine.

Table III. Cytokine-induced Activation of HIV-1 Expression

Pt. no.	$CD4^+$ count	Medium	PHA (4 μg/ml)	IL-2	IL-15
(*A*) Cytokine concentrations: 1, 5, and 10 ng/ml					
1	0	10(2)	13(3)	16(1)*	11(2)
2	0	< 8	< 5,000*	39(26)*	< 8
3	20	< 8	< 8	< 8	< 8
23	20	27(1)	76(12)*	249(149)	31(1)
24	60	16(5)	> 5,000*	< 5,000*	46(8)*
25	60	< 8	91(16)*	67(5)*	< 8
26	100	174(161)	5,198(1,237)*	2,046(2,811)	325(194)
27	130	13(2)	110(29)*	22(3)*	16(3)
28	200	13(2)	405(103)*	4,326(920)*	71(16)*
29	280	1,050(106)	> 5,000*	< 5,000*	744(87)
30	280	45(25)	396(24)*	119(27)*	129(114)
6	310	< 8	< 8	< 8	< 8
8	330	140(6)	312(5)*	138(74)	314(36)*
10	360	< 8	< 8	35(26)*	< 8
15	580	< 8	< 8	< 8	< 8
31	600	< 8	< 8	< 8	< 8
32	660	< 8	< 8	< 8	< 8
(B) Cytokine concentrations: 0.0001, 0.001, 0.01, 0.1, 1, 10, 100 and 200 ng/ml					
39	0	13(1)	125(9)*	315(12)*	30(8)
40	130	17(2)	60(3)*	42(4)*	18(4)
41	180	15(1)	36(6)*	67(6)*	21(1)*
42	260	< 8	< 8	< 8	< 8

PBMC were prepared from 21 HIV-1–infected subjects and cultivated in medium or medium supplemented with PHA or cytokine. Supernatants were harvested after 14-d culture and HIV-1 p-24 antigen was measured by ELISA. Data shown are mean(SD) of triplicate p-24 determinations (*A*) or duplicate determinations (*B*) expressed as pg/ml. p-24 levels in the presence of IL-2 and IL-15 are the peak values obtained among three concentrations used (1, 5, and 10 ng/ml) in *A* and eight concentrations used in *B* (0.0001, 0.001, 0.01, 0.1, 1, 10, 100, and 200 ng/ml). *Significant enhancement of HIV-1 expression is defined by a mean value of supernatant p-24 greater than baseline ($P < 0.05$ using Student's *t* test where an absolute value was obtained for both levels) or for experiments wherein one level was above or below the level of detection where the inducible level of p-24 antigen and the baseline level differed by more than the standard deviation of the value that was within the limit of detection of the assay.

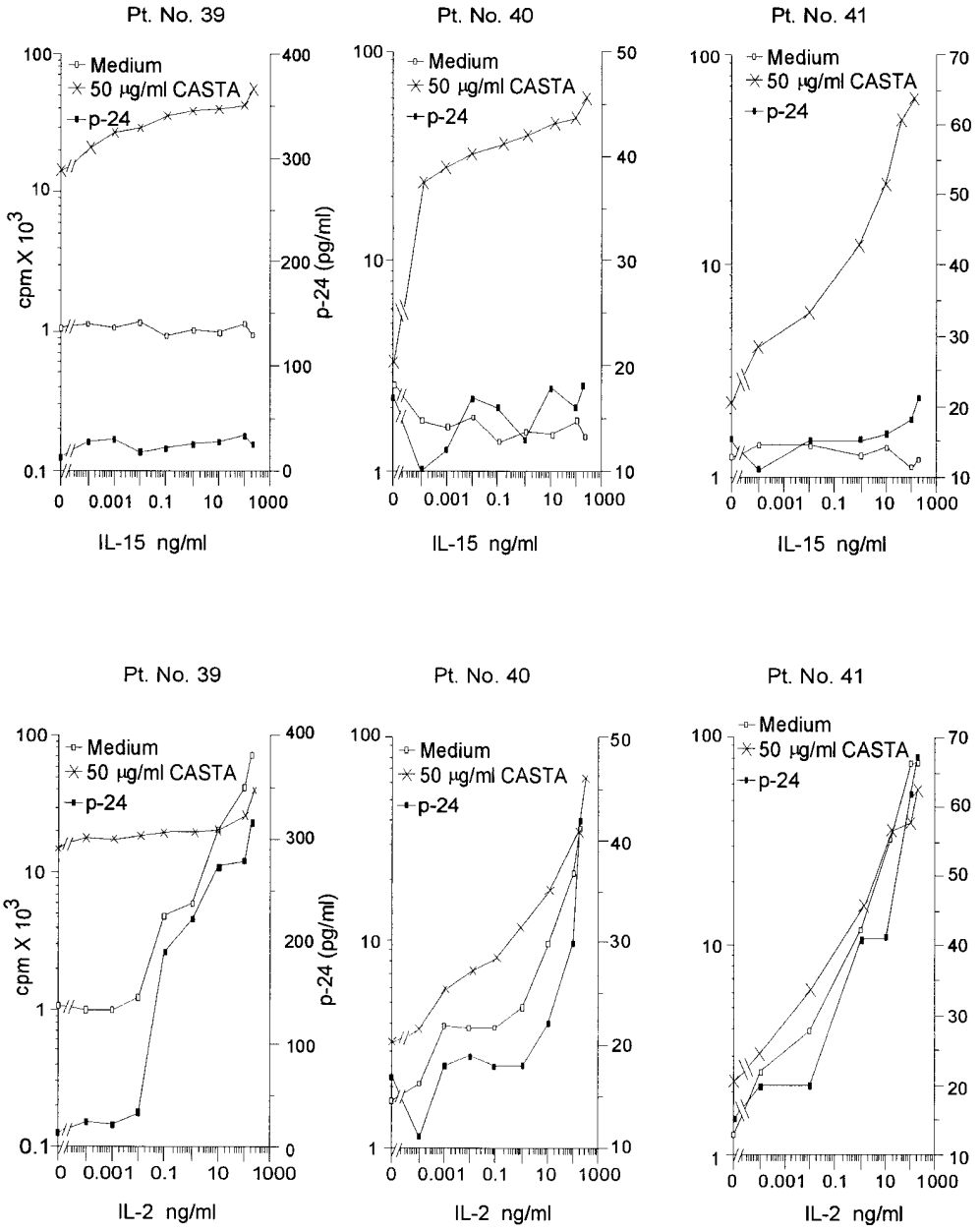

Figure 2. Cytokine effects on CASTA-induced and spontaneous lymphocyte proliferation and HIV-1 p-24 antigen production. PBMC prepared from HIV-1–infected subjects were cultivated with the indicated concentrations of IL-2 or IL-15 and were assayed for spontaneous and CASTA-induced lymphocyte proliferation after 7-d culture and for antigen-independent, cytokine-induced HIV-1 p-24 antigen production after 14-d culture.

As seen in earlier experiments, IL-15 had no effect on spontaneous proliferation or HIV-1 p-24 antigen production at concentrations up to 200 ng/ml.

Discussion

The in vitro activities of the newly identified cytokine IL-15 are similar to those of IL-2 (1). Like IL-2, IL-15 supports the growth of IL-2–dependent T cell lines and enhances proliferative responses to T cell mitogens by lymphocytes obtained from healthy controls (1). We have found that like IL-2, IL-15 enhances antigen-induced lymphoproliferative responses by PBMC obtained from HIV-1 infected patients. The magnitude of enhancement induced by IL-2 and especially by IL-15 tended to be greater among patients with relatively preserved immune responses (higher $CD4^+$ lymphocyte counts). Seder et al. recently demonstrated that IL-15 enhanced proliferative responses by PBMC and $CD4^+$ T cells obtained from HIV-1–infected individuals in response to antigens or mitogen (12), although the magnitude of augmentation was not related to $CD4^+$ T cell counts in those studies, and the effects of IL-2 and IL-15 on spontaneous proliferation and HIV-1 expression were not examined.

IL-2 enhanced not only antigen-induced lymphocyte proliferation but also activated freshly prepared lymphocytes to proliferate in the absence of other stimuli. In contrast, IL-15 had essentially no effect on [^{3}H]thymidine incorporation of unstimulated PBMC, but significantly enhanced lymphoproliferative responses to soluble antigens of Candida albicans and to streptokinase. Although IL-2 and IL-15 bind to the same β and γ subunits of the IL-2 receptor, IL-15 does not use the IL-2 receptor α chain for T cell activation (1). Thus, the α chain may be important in mediating the mitogenic effect of IL-2 or T cell receptor activation may be required to induce expression of

the IL-15 receptor α chain, leading to a proliferative effect of IL-15 only when antigen is engaged by the T cell receptor. We do not think that the failure of IL-15 to enhance spontaneous proliferation is related to a differential dose–response effect since concentrations of IL-15 > 2,000-fold greater than mitogenic concentrations of IL-2 failed to enhance spontaneous lymphocyte proliferation (Table II, Fig. 2).

Of potential relevance to the role of these cytokines in the treatment of HIV-1 infection, we found that IL-2 directly activated the expression of HIV-1 from resting PBMC obtained from 12 of 21 HIV-1–infected patients whose cells were examined in this assay. The magnitude of enhancement ranged from ~ 1.6-fold to > 300-fold. When data from all experiments were combined, cytokine-induced activation of HIV-1 expression was seen primarily among cultures from patients with more advanced immune deficiency as HIV-1 production was enhanced by IL-2 in 11 of 15 cultures from patients with ≤ 300 $CD4^+$ cells/μl and in only one of 6 cultures from patients with > 300 $CD4^+$ cells/μl. In contrast, IL-15 increased HIV-1 production in only 4 of 21 experiments and the magnitude of enhancement was modest (~ two- to fivefold). Since IL-2 may enhance the anti-HIV activities of $CD8^+$ T cells (13) the effects of IL-2 on HIV-1 induction may have been even more dramatic if purified $CD4^+$ T cell populations were examined in this assay. Experiments are in progress to evaluate the relative effects of IL-2 and IL-15 on HIV-1 expression by purified $CD4^+$ T cells and on $CD8^+$ T cell–mediated antiviral activities.

We suspect that activation of spontaneous HIV-1 expression in unseparated PBMC is related to the mitogenic effect of IL-2. As seen in Fig. 2, the magnitude of HIV-1 production induced by IL-2 closely mirrors the activation of spontaneous proliferation by this cytokine. Although in some experiments, lower concentrations of IL-2 enhanced antigen-induced proliferation without a mitogenic effect, in most instances the dose–response curves for antigen-induced proliferation and spontaneous proliferation were parallel as shown in Fig. 2.

Recently, Kovacs et al. reported that administration of high doses of recombinant IL-2 to patients with HIV-1 infection was associated with dramatic increases in the numbers of circulating $CD4^+$ lymphocytes and transient but substantial increases in plasma HIV-1 levels (10). As in the present in vitro studies, viral activation by IL-2 in vivo was most pronounced among patients with lower $CD4^+$ counts. Preliminary data suggest that these rises in circulating $CD4^+$ lymphocytes after IL-2 administration are associated with heightened spontaneous lymphocyte proliferation (14). Thus, the mitogenic effect of IL-2 may directly activate the proliferation, expansion, and induction of HIV-1 expression in lymphocytes of HIV-1–infected patients.

Our data suggest that IL-15 is not likely to induce nonspecific $CD4^+$ lymphocyte expansion. On the other hand, augmentation of impaired antigen-induced lymphocyte proliferative responses in HIV-1 infection without mitogenic activation of T cell proliferation or HIV-1 expression may prove a useful strategy for immune restoration in persons with HIV-1 infection.

Acknowledgments

The authors would like to acknowledge Mr. Daniel Georges and Mr. Rodney Schmelzer for their technical assistance.

These studies were supported by awards AI25879 and AI136129 from the National Institutes of Health.

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