Human Immunodeficiency Virus–infected Adherent Cell-derived Inhibitory Factor (p29) Inhibits Normal T Cell Proliferation through Decreased Expression of High Affinity Interleukin-2 Receptors and Production of Interleukin-2

Adlen Ammar,* Yasmina Sahraoui,* Andreas Tsapis,‡ Anne-Marie Bertoli,* Claude Jasmin,* and Vassilis Georgoulias*
**Oncogénèse Appliquée, Institut National de la Santé et de la Recherche Médicale (INSERM) U268, Hôpital Paul Brousse, 94804 Villejuif, France; and ‡Unité d'Immunopathologie, INSERM U108, Hôpital Saint Louis, 75010 Paris, France*

Abstract

Adherent cells from HIV-infected subjects as well as in vitro HIV-infected normal adherent cells produce spontaneously a 29-kD (p29) factor that inhibits mitogen-induced proliferation of normal T cells. p29 mediates a partial dose-dependent inhibition of total protein synthesis in both nonstimulated and PHA-activated cells that is associated with impaired PHA-induced expression of IL-2 receptor (IL-2R)α chain, HLA-class II molecules, and production of IL-2 by these cells; conversely, p29 does not modify the expression of IL-2Rβ chain, 4F2, CD9, or transferrin receptor, or the production of IL-1 and TNFα by the cells. 1 h preincubation of the cells with p29 is sufficient to detect its biologic activity and added rIL-2 abrogates p29-induced inhibition of IL-2Rα chain expression; however, p29 does not display any biologic effect on already expressed IL-2Rα chains. The impaired expression of IL-2Rα chain mediated by p29 is not due to a decreased accumulation of the corresponding mRNA transcripts, but is associated with a two-fold increase of intracellular cAMP. Binding experiments with ^{125}I-rIL-2 reveals that p29 induces a 50% decrease in the number of both high and low affinity IL-2R per cell. p29 also inhibits alloantigen-induced proliferation of PBMC, whereas it does not modify IL-2-dependent proliferation of 48-h PHA-blasts that already express high affinity IL-2R. These findings indicate that p29 mediates its biologic activity during early stages of T cell activation affecting the expression of high affinity IL-2R and production of IL-2, through a nontranscriptional mechanism involving an increase of intracellular cAMP. (*J. Clin. Invest.* 1992. 90:8–14.) Key words: human immunodeficiency virus • immunodeficiency • interleukin-2 • interleukin-2R • T cell activation

Introduction

Antigens or mitogens activate normal T cells which then express different novel molecules on their surface, such as IL-2 receptors (IL-2R),[1] CD9, CD69, 4F2, HLA-class II molecules (1–3) and produce a number of cytokines such as IL-2, IL-4, -IFN, etc., (4) which participate in cell cooperation. Cell activation is followed by cell proliferation which is regulated by the binding of IL-2 to its high affinity functional IL-2R (5–6).

Functional IL-2R has been shown to be composed of, at least, two different IL-2-binding chains: IL-2Rα chain (also named Tac molecule or p55) binds its ligant with a low affinity ($K_d = 10^{-12}$ M) (7) and IL-2Rβ (also named p75) which binds IL-2 with an intermediate affinity ($K_d = 0.2–1 \times 10^{-10}$ M) (8–10). The association of these chains is needed to form high affinity IL-2R (11–13). Monoclonal antibodies have been developed against these already isolated IL-2–binding polypeptides and their genes have been cloned and sequenced (14–17).

Infection by HIV is followed by severe impairment of cell-mediated immune responses that can be detected at all clinical stages of the disease (18–27). These abnormalities could be due to the decreased number of $CD4^+$ cells since these cells have a key role in both cell-mediated and humoral immunity against various antigens. Moreover, $CD4^+$ cells display various functional abnormalities which seem to be due to several mechanisms. Indeed, it has been reported that viral envelope protein gp120 can mediate an immunosuppressive effect on T cell responses, probably through impaired signal transduction (28–31). Moreover, HIV-infected cells can produce factors that inhibit normal T cell activation (32, 33) or IL-1 activity (34, 35).

We recently reported that adherent cells from HIV-infected subjects spontaneously produce an activity inhibiting the in vitro clonal proliferation of T cells from both patients and normal subjects through decreased production of IL-2 and expression of IL-2Rα chain (36). This inhibitory activity, which is also produced after in vitro HIV infection of normal adherent cells, was shown to be due to a thermosensible protein of 29 kD (p29) partially purified by chromatography, SDS-PAGE, and elution of the proteins from the gel (37). p29 is distinct from viral p24, gp120, and nef proteins as well as from α/β- and interferons, prostaglandin E2, tumor necrosis factor α, and transforming growth factor β (37). p29 could not be detected in media conditioned by adherent cells from normal HIV seronegative subjects and patients with other various viral, parasitic, or fungal diseases suggesting that secretion of this inhibitory factor is associated with HIV infection (37).

The cellular origin of p29 seems to indicate that this molecule could be involved in the regulation of normal immune response through modulation of the IL-2/IL-2R system. Thus, we investigated the effect of p29 on cell activation and, more precisely, on the IL-2R function. Our findings demonstrate that p29 can inhibit the expression of IL-2Rα, but not IL-2Rβ chain, inducing a decrease in number of both high and low affinity receptors per cell. In addition, p29 decreases IL-2 production by normal T cells, thus leading to an inhibition of

Address correspondence to Dr. V. Georgoulias, Institut National de la Santé et de la Recherche Médicale U268, Hôpital Paul Brousse, 14–16 Avenue P. V. Couturier, 94804 Villejuif, France.

Received for publication 1 April 1991 and in revised form 31 December 1991.

J. Clin. Invest.

0021-9738/92/07/0008/07 $2.00
Volume 90, July 1992, 8–14

1. *Abbreviations used in this paper:* IL-2R, IL-2 receptor; rIL-2, recombinant IL-2; TNFα, tumor necrosis factor α.

normal cell proliferation. These modifications of T cell growth are associated with a p29-mediated increase of intracellular cAMP accumulation that has recently been shown to modulate the expression of IL-2R chains (38–39).

Methods

Cells. PBMC were obtained from healthy blood donors by Ficoll-Paque (Pharmacia Fine Chemicals, Uppsala, Sweden) density (d = 1,077 g/liter) centrifugation of heparinized blood. Interface cells were resuspended in RPMI 1640 (Gibco Laboratories, Grand Island, NY) supplemented with 10% (vol/vol) heat-inactivated fetal calf serum (Gibco), 2 mM L-glutamine and antibiotics (1% vol/vol) (complete growth medium) at 10^6 cells/ml. Cells were activated for 48 h with PHA-P (0.1% vol/vol; Difco Laboratories Inc., Detroit, MI) (PHA-blasts) in the presence or the absence of p29 (0.25 μg/ml), which was biochemically partially purified by G25, gel filtration, and anion-exchange chromatography as reported previously (37), at 37°C in 5% CO_2 in air. Washed cells were recovered and stained with various monoclonal antibodies by indirect immunofluorescence and analyzed by flow cytometry (FACSscan®; Becton Dickinson Co., Mountain View, CA). Culture supernatants were filtered (0.22 μm; Millipore Corp., Bedford, MA) and stored at −20°C until use. In some experiments normal PBMC were preincubated for 1 h with p29 (0.25 μg/ml) at 37°C, and extensively washed cells were resuspended in fresh complete growth medium and activated with PHA-P as above.

Monoclonal antibodies. Expression of IL-2Rα chain (CD25) was evaluated by either the IOT14 (Immunotech, Luminy, France) or the anti-Tac (kindly provided by Dr. T. Waldmann, National Institutes of Health, Bethesda, MD) mAb whereas IL-2Rβ chain expression was analyzed using the TU-27 mAb (kindly provided by Dr. K. Sugamura, Tohoky University School of Medicine, Sendai, Japan; 17). HLA-class II molecules were detected using mAb 206 (a gift from Dr. Charron, Institut Biomedical des Cordeliers, Paris, France); transferrin receptors were analyzed using the IOT9 mAb (Immunotech) whereas 4F2 and CD9 molecules were detected by the 4F2 and ALB6 (a gift from Dr. C. Boucheix, INSERM U268, Villejuif, France) mAbs, respectively.

Determination of protein synthesis. Freshly isolated PBMC were cultured at 2×10^5 cells/200 μl per well in leucine- and serum-free M199 medium (Gibco) in the presence of various concentrations of p29 or 500 μM cycloheximide for 24 h. In parallel experiments cells were also stimulated with PHA-P. 2 μCi/well of [^{3}H]leucine (sp act 140 Ci/mmol; CEA, Saclay, France) were subsequently added and cells were incubated at 37°C for 4 h and/or 24 h. After automatic harvesting of the cultures, cell-associated radioactivity was measured by liquid scintillation.

Binding of ^{125}I-recombinant IL-2 (rIL-2). PBMC stimulated as above in the presence or the absence of partially purified p29 (0.25 μg/ml) for 48 h, were washed twice, incubated for 1 h in complete growth medium at 37°C, washed again, and counted. Binding of ^{125}I-rIL-2 was performed according to Robb et al. (7) with slight modifications (40). Briefly, 3×10^6 cells were incubated with serial dilutions of ^{125}I-rIL-2 (New England Nuclear, Boston, MA; sp act 20–50 μCi/μg) for 2 h at 4°C. Cell suspensions were then overlaid onto an oil mixture of dibutylphthalate and phthalic acid bis-(3,5,5 trimethylhexyl) ester (1.5:1, vol/vol, Sigma Chemical Co., St. Louis, MO) and centrifugated at 9,000 *g* for 2 min. The tips of the tubes containing the cell pellet and the cell supernatant were counted in a gamma counter (LKB Instruments Inc., Bromma, Sweden) to determine cell-bound and cell-free radioactivity. Nonspecific binding was determined in the presence of a 500-fold excess of unlabeled rIL-2 (Amgen Biologicals, Thousand Oaks, CA). The number of binding sites per cell and the association constant (K_d) were evaluated by Scatchard analysis.

Northern blot. Total cellular RNA was extracted from cells activated for 24 h as above using the LiCl2-urea method. 10 μg of total RNA was run on agarose gels using formaldehyde, blotted to Gene Screen membranes (Amersham International, Amersham, Bucks, UK), and hybridized under high stringency conditions with a nick-translated specific Tac (pIL2-R2; kindly provided by Dr. W. Greene, National Institutes of Health, Bethesda, MD; sp act 10^8 cpm/μg) or p70 (pUC 30SD; a gift from Dr. Taniguchi, University Medical School, Osaka, Japan) cDNA probes. The filters were exposed with an intensifying screen at −80°C using Kodak X-AR5 films. Subsequently, the same filters were extensively washed and rehybridized with a 1.9-kb β-actin cDNA probe (a gift from Dr. V. Zannis, Boston, MA).

Measure of intracellular cAMP. PBMC (10^6/ml) were incubated in the presence or the absence of p29 (0.25 μg/ml) and PHA for 15 min. Cells were centrifuged at 2,000 rpm for 2 min, resuspended in Tris buffer (10 nM Tris-Hcl, 1 nM $MgCl_2$, pH 7.4), and lysed by sonication. The cell lysate was placed in liquid nitrogen for 5 min and, subsequently, in a boiling water bath for 2 min. cAMP was measured in cell lysates using a radioimmunoassay kit (cAMP-^{125}I-RIA, Pasteur Diagnostics, Marnes-la-Coquette, Paris, France) according to the manufacturer's instructions. Control experiments were performed in the presence of forskolin (2 μM final concentration).

Cell proliferation. PBMC (2×10^5 cells/200 μl) were stimulated as above in the presence or the absence of semi-purified p29 (0.25 μg/ml). In addition, PBMC were cultured with 2×10^5 irradiated allogeneic PBMC under the same conditions as above but in the absence of PHA-P. Cultures were performed in 96 flat-bottomed microtest plates and incubated for 6 d at 37°C in 5% CO_2 in air. At the end of the culture period, cells were pulsed with 1 μCi/well of [^{3}H]thymidine (sp act 10–25 Ci/mmol; CEA) for an additional 16 h. Cells were recovered using a cell harvester and cell-associated radioactivity was counted by liquid scintillation. In some experiments, 48-h PHA-blasts were used to determine the effect of p29 on the IL-2–induced cell proliferation. For this purpose, washed cells were incubated in the presence of increasing concentrations of rIL-2 (Eurocetus; Emeryville, The Netherlands) in the absence or the presence of p29 (0.25 μg/ml). After a 48-h cell incubation, cell proliferation was determined by [^{3}H]thymidine incorporation as above.

IL-2 assay. IL-2 activity in media conditioned by PHA-activated cells in the presence of p29 was determined using cytotoxic T lymphocyte line (CTLL)-2 cells according to the method described by Mosman (41) as modified by Tada (42). Briefly, 50 μl of twofold dilutions of the supernatant to be tested were added per well in 96 flat-bottomed microplates. Then, 6×10^3 CTLL-2 cells, resuspended in complete growth medium supplemented with 10^{-4} M 2-mercaptoethanol, were added to each microwell. Microplates were incubated at 37°C in 5% CO_2 in air for 24 h and viable proliferating cells were detected by adding 20 μl/well of bromure 3-(4,5 dimethylthiazol)-2,5-diphenyltetrazolium (MTT; Sigma Chemical Co.). 100 μl of 10% SDS-HCl 0.01 M were added for an additional 4–6 h. Controls included twofold dilutions of 25 French U/ml rIL-2 (1 French unit of IL-2 = 40 Biological Response Modifying Program units), complete growth medium, and complete growth medium supplemented with twofold dilutions of rIL-2. Absorbance was monitored at 570 nm.

Dosage of tumor necrosis factor α (TNFα) and IL1. Production of TNFα by activated PBMC was determined in conditioned media using a commercial immunoenzymatic assay (Biokine; T-cell Sciences, Inc., Cambridge, MA) according to the manufacturer's instructions. IL-1 activity in the same conditioned media was determined using the IL-1–dependent D10.G4 murine cell line. For this purpose, D10.G4 cells were washed, incubated in complete serum-free growth medium for 1 h at 37°C, washed again and seeded (10^4 cells/ml) in flat-bottomed microplates in the presence of twofold dilutions of 25 U/ml human rIL-1 (Boehringer Mannheim GmbH, Mannheim, FRG) or in the presence of similar dilutions of the supernatant to be tested. Additional controls were included as described for IL-2 dosage. Cultures were incubated for 48 h and pulsed with 1 μCi/well [^{3}H]thymidine for an additional 16 h. Cell-associated radioactivity was measured by liquid scintillation.

Results

Effect of p29 on protein synthesis. We initially investigated whether p29 can inhibit the overall protein synthesis by PHA-

Table I. Effect of p29 on Total Protein Synthesis

	p29	Time of incubation 4 h	24 h
RPMI	−	9.000±465*	ND
	+	7.662±623 (15%)‡	ND
PHA	−	13.359±762	88.319±543
	+	9.998±489 (25%)	56.700±987 (36%)
CXM	−	1.783±187	4.736±345
CXM + PHA	−	1.576±153	3.569±568

Normal PBMC were incubated in the presence or the absence of p29 (0.25 μg/ml) and PHA-M (1%, vol/vol), or 500 μM cycloheximide (CHX), for either 4 h or 24 h in leucine- and serum-free growth medium. Cells were, subsequently, pulsed with [^{3}H]leucine as described in Methods. * Results are expressed as the mean cpm±SD value from three different experiments. ‡ Numbers in parentheses, percentage of protein synthesis inhibition.

activated normal PBMC. Table I shows that incubation of PBMC with p29 inhibited by 15 and 20% the incorporation of [^{3}H]leucine at 4 h and 24 h, respectively. Moreover, p29 inhibited protein synthesis by PHA-activated cells (Table I). The effect of p29 on the overall cellular protein synthesis was dose dependent (not shown). Conversely, when cells were incubated in the presence of cycloheximide, a well-known inhibitor of protein synthesis, the inhibition of [^{3}H]leucine incorporation was >80% in all experiments (Table I).

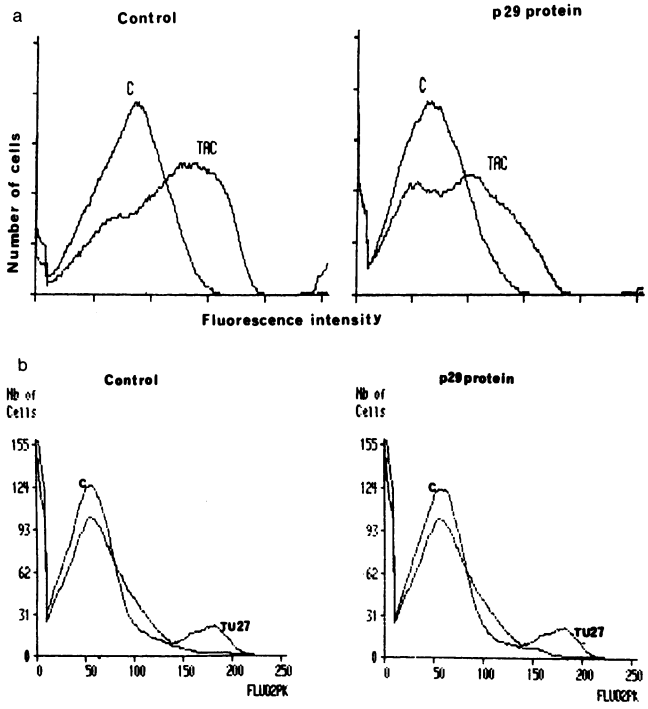

Figure 1. p29 inhibits the mitogen-induced expression of IL-2Rα but not of IL-2Rβ chain on normal PBMC. Normal PBMC (10^6/ml) were stimulated with PHA-P in the presence or the absence of biochemically semi-purified p29 (0.25 μg/ml) for 48 h, washed cells were stained with either IOT14 (*a*) or TU27 (*b*) mAb by indirect immunofluorescence and were analyzed by flow cytometry.

Table II. Effect of p29 on the Expression of Activation-associated Molecules

Molecule	RPMI 1640	p29	Percentage of inhibition
IL2-Rα (CD25)	65±8*	44±7	32±11
IL2-Rβ	15±8	14±8	0
HLA-class II	52±3	32±6	34±11
Transferrin-receptor	38±6	34±3	8±8
4F2	85±8	79±2	3±4
CD9	64±3	60±5	2±3

Normal PBMC (10^6/ml) were stimulated with PHA-P (0.1% vol/vol) in the presence or the absence of p29 (0.25 μg/ml) for 48 h and washed cells were stained with appropriate mAb by indirect immunofluorescence and analyzed by flow cytometry. * Results are expressed as the mean±SD values of the percentage of positively stained cells from six different experiments.

Effect of p29 on the expression of activation-associated molecules. Since p29 mediates a partial inhibition of protein synthesis, it was of interest to investigate whether this factor displays a specific effect on the expression of different molecules or the production of various cytokines produced during cell activation. Fig. 1 demonstrates that p29 could inhibit the expression of IL2-Rα (p55) but not of IL-2Rβ (p70) chains. It is interesting to note that the biphasic pattern of p29-mediated effect on the expression of IL-2Rα chain (Fig. 1) suggests that p29-mediated inhibitory activity is confined to a subpopulation of PBMC. Moreover, p29 induced an inhibition of HLA class-II molecules expression (Table II). Conversely, p29 did not modulate the expression of transferrin receptor, 4F2 and CD9 molecules. 1 h preincubation of normal PBMC with p29 at 37°C was sufficient to mediate its biologic activity (Table III) whereas incubation of 48-h PHA-blasts with this factor did not modulate IL-2Rα chain expression on these cells (Table III). Exogenously added rIL-2 abrogated the inhibitory effect of p29 on the IL-2Rα chain expression in a dose-dependent manner (Fig. 2).

Table III. Effect of p29 Short Cell Exposure on the IL-2Rα Chain Expression

Cells	p29	Time of exposure 1 h	24 h
PBMC	−	42±12*	46±9
PBMC	+	26±7 (38%)‡	24±8 (48%)
PHA-blasts	−	74±16	63±10
PHA-blasts	+	72±14 (3%)	66±13 (0%)

Normal nonstimulated PBMC as well as 48-h PHA-blasts (10^6/ml) were incubated with either complete growth medium or p29 (0.25 μg/ml) for 1 h and 24 h at 37°C. Then, extensively washed cells were reseeded in fresh complete growth medium and cultured for an additional 48 h with PHA-P. Subsequently, cells were stained with the 10T14 mAb by indirect immunofluorescence and analyzed by flow cytometry. * Results are expressed as mean±SD values of percentage of positively stained cells from four different experiments. ‡ Mean percentage of inhibition of IL-2Rα expression.

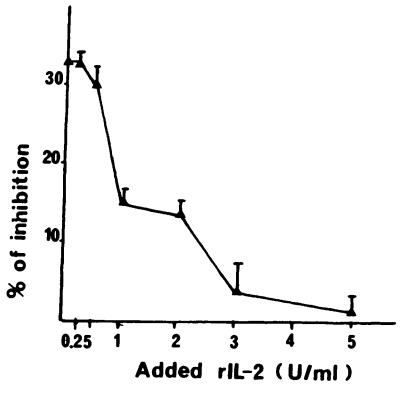

Figure 2. Effect of exogenous rIL-2 on p29-mediated inhibition of IL-2R chain expression. Normal PBMC (10^6/ml) were activated with PHA-P (0.1% vol/vol) in the presence of p29 (0.25 μg/ml) for 48 h. Subsequently, washed cells were incubated with increasing concentrations of rIL-2 for an additional 24 h. Immunofluorescence studies were performed using the IOT14 mAb and cells were analyzed by flow cytometry. The results represent the mean values of four different experiments. The mean percentage of IOT14$^+$ cells in the absence and the presence of p29 was 67 and 42%, respectively.

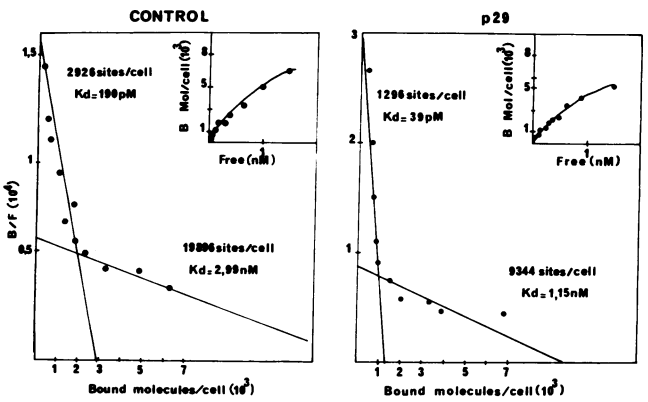

Figure 3. Binding of ^{125}I-rIL-2 on cells activated in the presence of p29. Normal PBMC (10^6/ml) were activated with PHA-P in the presence or the absence of p29 (0.25 μg/ml) for 48 h and extensively washed cells were used for binding of ^{125}I-rIL-2 under high and low affinity conditions as described in Methods. The number of binding sites and the dissociation constant were evaluated by Scatchard analysis.

In parallel experiments we studied the production of some cytokines by cells stimulated in the presence of p29. Table IV shows that p29 inhibited by 50% the production of IL-2 whereas it did not inhibit the production of IL-1 and TNFα; on the contrary, p29 slightly increased the secretion of these cytokines (Table 4).

p29 induces a decrease of high and low affinity IL2-R/cell. Subsequently, we studied the affinity of IL-2R expressed on normal stimulated T cells cultured in the presence of p29. Fig. 3 indicates a representative experiment of ^{125}I-rIL2 binding on these cells showing a twofold decrease of the number of high affinity IL-2–binding sites per cell (1,296 sites/cell) compared to cells stimulated in the absence of p29 (2,926 sites/cell). It should be noted that p29 increased the affinity of IL-2R in comparison to normal controls (K_d = 39 pM vs 190 pM, respectively; Fig. 3). In addition, Fig. 3 demonstrates that p29 also induced a decrease of the number of low affinity IL-2–binding sites per cell, which is known to correspond to membrane-expressed IL-2Rα molecules; this observation corroborates with the described p29-mediated decreased expression of this molecule.

p29 cannot inhibit the accumulation of IL2-Rα and IL2-Rβ mRNA. To investigate the mechanisms of p29-induced inhibition of IL-2Rα chain expression, total RNA from cells incubated in the presence or absence of p29 were hybridized with IL-2Rα and IL-2Rβ cDNA probes. Kinetic studies show that p29 did not modify the accumulation of IL-2Rα mRNA transcripts (Fig. 4). Although the rehybridization of the same filters with a β-actin cDNA probe revealed that there were some differences on the quantity of RNA loaded to the gels, the ratio of the unique band of 1.9 kb between the control and experimental points was the same, indicating that there is no significant effect of p29 on IL-2Rα mRNA accumulation. Fig. 5 also demonstrates that p29 has no effect on the accumulation of IL-2Rβ specific mRNA transcripts.

p29 induces the accumulation of intracellular cAMP. cAMP has been reported to inhibit cell proliferation (43–45) and, recently, has been shown to posttranscriptionally modulate the expression of both IL-2–binding chains (38–39). Thus, we studied the effect of p29 on the accumulation of intracellular cAMP. Table V demonstrates that p29 induced a twofold increase of the intracytoplasmic content of cAMP in nonstimulated and PHA-activated normal PBMC. Forskolin, an established intracellular cAMP-inducing agent, induced a 3.5-fold

Table IV. Dosage of Cytokines in Media Conditioned by PHA-activated PBMC in the Presence of p29

Cytokine	p29	Concentration
IL2	−	3±2 U/ml
IL2	+	1.5±0.2 U/ml
IL1	−	0.5±0.1 ng/ml
IL1	+	2±0.2 ng/ml
TNFα	−	158±25 pg/ml
TNFα	+	335±166 pg/ml

Normal PBMC (10^6/ml) were stimulated with PHA-P in the presence or the absence of p29 (0.25 μg/ml) for 48 h and cell-free supernatants were tested for IL-1, IL-2, and TNFα as described in Methods. Results represent the mean±SD values observed from three different experiments.

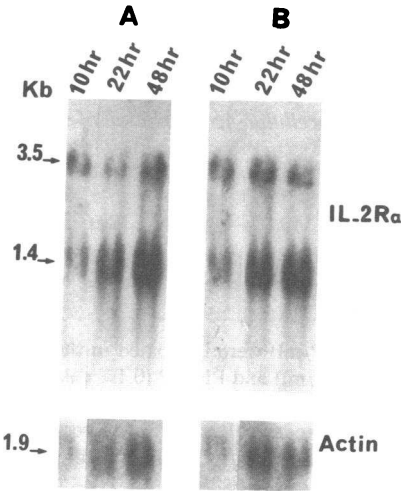

Figure 4. Kinetic of IL-2Rα chain mRNA expression. Normal PBMC (10^6/ml) were activated with PHA-P for 10, 22, or 48 h in the absence (*A*) or presence (*B*) of p29 (0.25 μg/ml). Extracted total RNA was analyzed by Northern blot using an IL-2Rα cDNA probe. The same filters were washed and rehybridized with a β-actin cDNA probe.

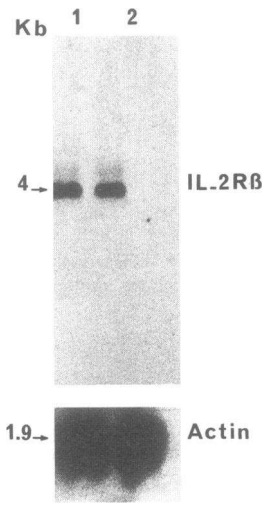

Figure 5. p29 does not modify the accumulation of IL-2Rβ mRNA transcripts. Normal PBMC (10^6/ml) were activated with PHA-P for 24 h in the absence (lane *1*) or the presence (lane *2*) of p29 (0.25 μg/ml) for 24 h, and extracted total RNA was analyzed by Northern blot using an IL-2Rβ cDNA probe as described in Methods. Subsequently, the same filters were extensively washed and rehybridized with a β-actin cDNA probe.

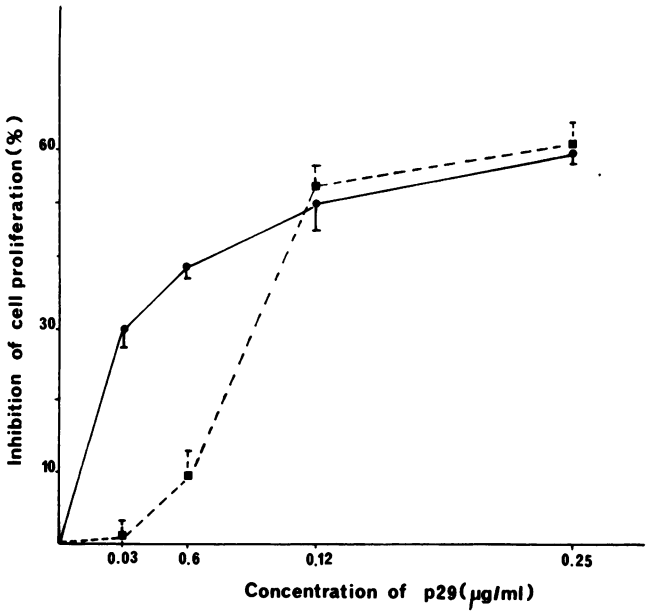

Figure 6. Proliferation of normal PBMC in the presence of p29. Normal PBMC (10^6/ml) were activated with PHA-P (0.1% vol/vol) (——) in the presence or the absence of biochemically semi-purified p29 (0.25 μg/ml) for 6 d and cell-associated radioactivity was determined by [^{3}H]thymidine uptake during the last 16 h of the culture. In addition, PBMC (2×10^6/ml) (-----) were activated with allogeneic irradiated (2,500 rads) PBMC (2×10^6/ml) in the absence or the presence of p29 and cell proliferation was determined as previously. The results, which represent mean±SD values of six different experiments, are expressed as the percentage of inhibition of cell proliferation.

higher content of intracellular cAMP than p29 (Table V). This effect of p29 on the accumulation of intracellular cAMP was dose dependent reaching a plateau at relatively low concentrations of p29 (0.12 μg/ml; not shown).

Effect of p29 on cell proliferation. Since p29 inhibits both the expression of high IL-2R and IL-2 production by activated T cells we studied its effect on mitogen- and alloantigen-induced cell proliferation of normal cells. In several experiments performed p29 could not inhibit the proliferation of PHA-activated cells in a 72-h proliferation assay. Conversely, a clear inhibition of cell proliferation mediated by p29 could be detected in a 6-d assay. Indeed, Fig. 6 indicates an inhibition of cell proliferation in the presence of p29, irrespectively of the stimulus; however, it is noteworthy that inhibition was never more than 60% compared to control values. The addition of rIL-2 at the beginning of the culture could abrogate the inhibitory effect of p29 on PHA-induced cell proliferation (not shown), suggesting that p29-mediated inhibition of cell proliferation is associated with the impaired production of IL2. In addition, p29 could not modify the IL-2–dependent proliferation of normal PHA-blasts (Table VI).

Discussion

The results presented here demonstrate that p29 inhibitory factor, produced spontaneously by HIV-infected adherent cells (37), partially inhibits the IL-2/IL-2-R system on mitogen-activated normal T cells thus explaining the decreased mitogen- and antigen-induced cell proliferation mediated by this molecule. Indeed, immunofluorescence studies have shown that p29 induces an inhibition of IL-2Rα but not of IL-2Rβ chain expression. This observation was confirmed by equilibrium binding experiments which demonstrate a decreased number of both high and low affinity IL-2–binding sites. It is known that high affinity IL-2R are composed of a heterodimer of associated IL-2Rα and IL-2Rβ polypeptides whereas low affinity IL-2R represent only IL-2Rα chain (11–17). However, binding experiments showed that although p29 decreases the number of binding sites per cell, it induces a decrease of the dissociation constant of both high and low affinity IL-2R (Fig. 3). This

Table V. Effect of p29 on the Intracellular Accumulation of cAMP

Cell stimulation with	−p29	+p29
RPMI	11±8*	22±6
PHA-P	9±8	20±11
Forskolin	75±10	ND

Normal unfractionated PBMC (10^6/ml) were incubated in the presence or the absence of p29 (0.25 μg/ml) and PHA-P (0.1% vol/vol) or forskolin (2 μM/ml) for 15 min. Intracellular cAMP was determined by RIA as described in Methods. * Results are expressed as mean±SD values of intracellular cAMP (pg/ml) from five different experiments.

Table VI. Effect of p29 on the IL-2–dependent Proliferation of PHA-blasts

	rIL-2			
	U/ml			
	0	0.25	0.5	1
RPMI	7286±550*	12856±1205	20136±2020	35500±1990
p29	7017±620	13926±1520	24786±3550	36548±1505

PHA-blasts (10^6/ml) were cultured with increased concentrations of rIL-2 in the presence or the absence of p29 (0.25 μg/ml) and cell proliferation was determined by cell-associated [^{3}H]thymidine uptake as described in Methods. * Results are expressed as mean±SD of cpm/2×10^5 cells from five different experiments.

observation is difficult to explain in the light of our experimental data, but a rather simplistic explanation could be that p29-mediated inhibition of IL-2Rα chains facilitates the association of the remaining molecules with the normally expressed IL-2Rβ and shifts the equilibrium toward the formation of homodimers with increased binding affinity for IL-2. Additional studies would be necessary to evaluate the conformational association of IL-2 binding chains in the presence or the absence of p29.

Proliferation studies revealed that p29 could inhibit PHA-induced [^{3}H]thymidine uptake in a 6- but not a 3-d culture assay. This observation is probably due to the fact that p29 mediates a partial inhibition of both high affinity IL-2R expression and production of IL-2 by activated cells. Thus, under these conditions the expressed IL-2R and the secreted IL-2 are sufficient to sustain a nearly normal proliferation at a 3-d assay but the consumption of secreted IL-2 during the first days of culture induces a decreased cell proliferation in more prolonged cultures. This hypothesis is strongly supported by the observation that exogenously added rIL-2 could abrogate p29-mediated inhibition of cell proliferation and IL-2Rα chain expression.

Several studies showing that intracytoplasmic cAMP can inhibit cell activation of some T cells subsets (38, 39, 43–45), led us to investigate the effect of p29 on the accumulation of intracellular cAMP. Our findings demonstrate that p29 induces a dose-dependent increase of cAMP cellular content even in the absence of any mitogenic stimulus. In addition, in a previous study we have demonstrated that the main cell targets of p29 are CD8+ cells (Ammar, A., et al., submitted for publication), which have been shown to be sensitive to increased concentrations of cAMP (38). These observations which suggest that, at least partly, p29 mediates its inhibitory activity by increasing intracellular cAMP, is in agreement with recent studies indicating that cAMP induces a posttranscriptional inhibition of IL-2Rα chain expression. However, Johnson and Smith (38) have reported that increased concentrations of cAMP induce a posttranscriptional inhibition of IL-2Rβ chain and decrease of high affinity IL-2R through a rapid internalization and decreased rate of expression of new receptors; conversely, they did not observe any effect of cAMP on the expression of IL-2Rα chain. The contrasting effects of cAMP on the expression of IL-2Rα chains reported in our study and the literature could be attributed to methodological reasons, since our experiments were always performed with resting cells and p29 induced only a twofold increase of intracytoplasmic cAMP, whereas in the above mentioned study significantly higher concentrations of cAMP were used in experiments with cells already expressing high affinity IL-2R.

Activation of T cells is associated with novel expression of various molecules. Our findings indicate that p29 displays a selective inhibitory effect on the expression of IL-2Rα chain and HLA-class II molecules. This effect of p29 is not due to a general inhibition of protein synthesis of the cells since this molecule could not inhibit the expression of different activation-associated molecules (i.e., 4F2, CD9, transferrin receptor) as well as the production of some cytokines such as IL-1 and TNFα. In addition, Northern analysis using both IL-2Rα and IL-2Rβ chain cDNA probes showed that p29 could not modify the accumulation of specific mRNA transcripts. Kinetic studies of IL-2Rα chain mRNA expression also showed the absence of a p29-mediated effect, suggesting that this finding is not due to a modified mRNA stability. The rehybridization of the filters with a β-actin cDNA probe demonstrated that the ratio of intensity between control and experimental points was practically the same at all time points, we conclude that p29 does not modulate IL-2Rα chain expression at a transcriptional level. Taken together, these findings seem to indicate that p29 mediates its biological effect either at a translational level or/and during the migration of normally synthesized proteins to the cell membrane. Moreover, our observations strongly suggest that the mechanisms controlling the expression of activation-associated molecules or the production of various cytokines present a differential sensitivity to p29. Whether this phenomenon is due to a differential sensitivity of the above mentioned mechanisms to p29-mediated accumulation of intracytoplasmic cAMP is an interesting question. Since 1-h cell preincubation with p29 was sufficient to detect its biologic activity and membrane expression of IL-2Rα chain is a relatively early event during T cell activation, appearing at $\sim$ 6–8 h after cell activation (5–6), it is reasonable to hypothesize that p29 biologic activity is mediated at some precocious stages of cell activation. Conversely, p29 could not modify IL-2–dependent cell proliferation of PHA-blasts suggesting that p29 did not possess an anti-IL-2 activity. These observations taken together with the p29-mediated increase of intracytoplasmic cAMP, strongly suggest that early accumulation of cAMP can prevent some cell functions important for cell activation.

Previous studies have shown that HIV infection of T cells induces an abnormal signal transduction and inhibition of IL-2Rα gene expression (26, 29, 46, 47). In our study, we demonstrated that p29 production by HIV-infected adherent cells, both in vivo and in vitro (36, 37), downregulates the expression of high affinity IL-2R through posttranscriptional impaired membrane expression of IL-2Rα chain. Thus, HIV-induced production of p29 protein should be an additional mechanism involved in the impaired proliferation capacity of T cells during HIV infection. Probably, these mechanisms act in synergy, thereby explaining the profound T cell functional abnormalities observed even at early stages of HIV infection when only a small percentage of T cells are expressing the virus.

Acknowledgments

This work was supported by grants from the Association des Nouvelles Recherches Biomedicales and Mr. P. Castell.

References

1. Lipkowitz, S., W. C. Greene, A. L. Rubin, A. Novogrodsky, and H. K. Stenzel. 1984. Expression of receptors for interleukin 2: role in the commitment of T lymphocytes to proliferate. *J. Immunol.* 132:31–37.

2. Lumadue, J. A., A. B. Glick, and F. H. Ruddle. 1987. Cloning, sequence analysis, and expression of the large subunit of the human lymphocyte activation antigen 4F2. *Proc. Natl. Acad. Sci. USA.* 84:9204–9208.

3. Neckers, L. M., and J. Cossman. 1983. Transferrin receptor induction in mitogen-stimulated human T lymphocytes is required for DNA synthesis and cell division and is regulated by interleukin-2. *Proc. Natl. Acad. Sci. USA.* 80:3494–3498.

4. Efrat, S., S. Pilo, and R. Kaempfer. 1982. Kinetics of induction and molecular size of mRNA encoding human interleukin 2 and -interferon. *Nature (Lond.).* 297:236–239.

5. Cantrell, D. A., and K. A. Smith. 1983. Transient expression of interleukin-2 receptors: consequences for T cell growth. *J. Exp. Med.* 158:1895–1911.

6. Cantrell, D. A., and K. A. Smith. 1984. The interleukin-2 T-cell system, a new cell growth model. *Science (Wash. DC).* 224:1312–1316.

7. Robb, R. J., W. C. Greene, and C. M. Rusk. 1984. Low and high affinity cellular receptors for interleukin-2: implications for the level of Tac antigen. *J. Exp. Med.* 160:1126–1146.

8. Tsudo, M., R. W. Kozak, C. K. Goldman, and T. A. Waldmann. 1986. Demonstration of a non-Tac peptide that binds interleukin-2: a potential participant multichain interleukin-2 receptor complex. *Proc. Natl. Acad. Sci. USA.* 83:9694–9698.

9. Sharon, N., R. D. Klausner, B. R. Cullen, R. Chizzonite, and W. J. Leonard. 1986. Novel interleukin-2 receptor subunit detected by cross-linking under high affinity conditions. *Science (Wash. DC).* 234:859–863.

10. Sharon, N., J. P. Siegel, G. Tosato, J. Yodoi, T. L. Gerrard, and W. J. Leonard. 1988. The human interleukin-2 receptor β chain (p70). Direct identification, partial purification and patterns of expression on peripheral blood mononuclear cells. *J. Exp. Med.* 167:1265–1270.

11. Lowenthal, J. M., and W. C. Greene. 1987. Contrasting interleukin 2 binding properties of the α (p55) and β (p70) protein subunits of the human high affinity interleukin 2 receptors. *J. Exp. Med.* 166:1156–1161.

12. Dukovitch, M., Y. Wano, Lê Thi Bich-Thuy, P. Katz, B. R. Cullen, J. H. Kehrl, and W. C. Greene. 1987. A second human interleukin 2 binding protein that may be a component of high affinity interleukin 2 receptors. *Nature (Lond.)* 327:518–522.

13. Smith, K. A. 1987. The structure of high affinity IL2 receptor: α chains and β chains. *Immunol. Today.* 8:11–15.

14. Leonard, W. J., J. M. Depper, C. R. Grabtree, S. Rydikoff, J. Pumphrey, R. J. Robb, M. Krönke, P. B. Sveltik, N. J. Peffer, T. A. Waldmann, and W. C. Greene. 1984. Molecular cloning and expression of cDNAs for the human interleukin-2 receptor. *Nature (Lond.).* 311:631–635.

15. Hatakeyama, M., M. Tsudo, S. Minamoto, T. Kono, T. Doi, T. Miyata, M. Miyasaka, and T. Taniguchi. 1989. Interleukin-2 receptor β chain gene: generation of three receptor forms by cloned human α and β chain cDNAs. *Science (Wash. DC).* 244:551–554.

16. Leonard, W. J., J. M. Depper, T. Uchiyama, K. A. Smith, T. A. Waldmann, and W. C. Greene. 1982. A monoclonal antibody that appear to recognize the receptor for human T cell growth factor: partial characterization of the receptor. *Nature (Lond.).* 300:267–269.

17. Takeshita, Y., Y. Goto, K. Tada, K. Nagata, H. Asao, and K. Sugamura. 1989. Monoclonal antibody defining a molecule possibly identical to p75 subunit of interleukin-2 receptor. *J. Exp. Med.* 169:1323–1332.

18. Fauci, A. S., A. M. Macher, D. L. Longo, A. H., Rook, H. Masur, and E. P. Gelmann. 1984. NIH Conference: Acquired Immunodeficiency Syndrome: epidemiologic, clinical, immunologic and therapeutic considerations. *Ann. Intern. Med.* 100:92–106.

19. Gurley, R. J., K. Ikenchi, R. A. Byrn, K. Anderson and J. E. Groopman. 1989. $CD4^+$ lymphocyte function with early human immunodeficiency virus infection. *Proc. Natl. Acad. Sci. USA.* 86:1993–1997.

20. Ciobanue, N., K. Welbe, G. Gruger, S. Venuta, J. Gold, S. P. Veldman, C. Y. Wang, B. Kozimer, M. A. S. Moore, B. Safal, and R. Mertelsmann. 1983. Defective T cell response to PHA and mitogenic monoclonal antibodies in male homosexuals with acquired immunodeficiency syndrome and its in vitro correction by interleukin 2. *J. Clin. Immunol.* 3:332–336.

21. Lane, H. C., J. M. Depper, W. C. Greene, G. Whalen, T. A. Waldmann, and A. S. Fauci. 1985. Quantitative analysis of immune function in patients with acquired immunodeficiency syndrome. *N. Engl. J. Med.* 313:79–84.

22. Fauci, A. S. 1988. The human immunodeficiency virus: infectivity and mechanisms of pathogenesis. *Science (Wash. DC).* 239:617–622.

23. Diamond, D. C., B. P. Sleckman, T. Gregory, L. A. Lasky, J. L. Grenstein, and S. J. Burakoff. 1988. Inhibition of $CD4^+$ T cell function by the HIV envelope protein gp120. *J. Immunol.* 141:3715–3717.

24. Folks, T. M., J. Justement, A. Kinter, C. A. Dinarello, and A. S. Fauci. 1987. Cytokine-induced expression of HIV-1 in chronically infected promonocyte cell line. *Science (Wash. DC).* 238:800–802.

25. Petit, C. A. J., M. Tersmette, F. G. Terpstra, R. E. T. de Goede, R. A. W. W. van Lier, and F. Miedema. 1988. Decreased accessory function by human monocytic cells after infection with HIV. *J. Immunol.* 140:1485–1489.

26. Hoffmann, B., E. Langhoff, B. O. Linchardt, N. Odum, J. J. Hyldig-Nielsen, L. P. Ryder, P. Platz, B. K. Jakobsen, K. Bendtzen, N. Jakobsen, et al. 1989. Investigation of immunosuppressive properties of inactivated human immunodeficiency virus and possible neutralization of this effect by some patient sera. *Cell. Immunol.* 121:336–348.

27. Laurence, J., S. M. Friedman, E. K. Chatarach, M. K. Crow, and D. N. Posnett. 1989. Human immunodeficiency virus infection of helper T cell clones. Early proliferative defects despite intact antigen-specific recognition and interleukin 4 secretion. *J. Clin. Invest.* 84:1843–1848.

28. Hoxie, J. A., J. D. Alpers, J. L. Rackowski, K. Huebner, B. S. Haggarty, A. J. Cedarbaum, and J. C. Reed. 1986. Alterations in T4 (CD4) protein and mRNA synthesis in cells infected with HIV. *Science (Wash. DC).* 234:1123–1317.

29. Oyaizn, N., N. Chirmule, V. S. Kalyanaraman, W. W. Hall, R. A. Good, and S. Pahwa. 1990. Human immunodeficiency virus type 1 envelope glycoprotein gp120 produces immune defects in $CD4^+$ T lymphocytes by inhibiting interleukin-2 mRNA. *Proc. Natl. Acad. Sci. USA.* 87:2379–2383.

30. Weinhold, K. J., H. K. Lyerly, S. D. Stanley, A. A. Austin, T. J. Matthews, and D. P. Bolognesi. 1989. HIV1-gp120 mediated immune suppression and lymphocyte destruction in the absence of viral infection. *J. Immunol.* 142:3091–3097.

31. Linette, G. P., R. J. Hartzman, J. A. Ledbetter, and C. H. June. 1989. HIV-1 infected T cells show selective signaling defect after perturbation of CD3/antigen receptor pathway. *Science (Wash. DC).* 241:573–576.

32. Laurence, J., A. B. Gottlieb, and H. G. Kungel. 1983. Soluble suppressor factors in patients with acquired immune deficiency syndrome and its prodrome. *J. Clin. Invest.* 72:2072–2081.

33. Laurence, J., and L. Meyer. 1984. Immunoregulatory lymphokines of T-hybridomas from AIDS patients: constitutive and inducible suppressor factors. *Science (Wash. DC).* 225:66–69.

34. Berman, M. A., C. L. Sandborg, B. S. Calabria, B. S. Andrews, and G. J. Friou. 1987. Interleukin 1 inhibitor masks high interleukin-1 production in acquired immunodeficiency syndrome (AIDS). *Clin. Immunol. Immunopathol.* 42:133–140.

35. Locksley, R. M., S. Growe, M. D. Sadick, F. P. Heinzel, K. D. Gardner, Jr., M. S. McGrath, and J. Mills. 1988. Release of interleukin-1 inhibitory activity (contra-IL1) by human monocyte-derived macrophages infected with human immunodeficiency virus in vitro and in vivo. *J. Clin. Invest.* 82:2097–2105.

36. Lunardi-Iskandar, Y., V. Georgoulias, D. Vittecoq, M. T. Nugeyre, A. Ammar, C. Clemenceau, F. Barré-Sinoussi, J. C. Chermann, L. Schwartzenberg, and C. Jasmin. 1987. Peripheral blood adherent cells from AIDS patients inhibit normal T-colony growth through decreased expression of interleukin-2 receptors and production of interleukin-2. *Leuk. Res.* 11:753–760.

37. Ammar, A., C. Cibert, A. M. Bertoli, V. Tsilivakos, C. Jasmin, and V. Georgoulias. 1991. Biological and biochemical characterization of a factor produced spontaneously by adherent cells of HIV-infected patients inhibiting Tac expression on normal T cells. *J. Clin. Invest.* 87:2048–2055.

38. Johnson, K. W., and K. A. Smith. 1990. cAMP regulation of IL-2 receptor expression. Selective modulation of the p75 subunit. *J. Immunol.* 145:1144–1151.

39. Iwaz, I., E. Kouassi, T. S. Lafon, and J. P. Revillard. 1990. Elevation of cyclic adenosine monophosphate levels independently down regulates IL1, IL2 receptor synthesis. *Int. J. Immunopharmacol.* 12:631–637.

40. Alouche, M., Y. Sahraoui, Y. Augery-Bourget, Y. Ohashi, K. Sugamura, C. Jasmin, and V. Georgoulias. 1989. Presence of a p70 IL-2-binding peptide on leukemic cells from various hemopoietic lineages. *J. Immunol.* 143:2223–2229.

41. Mosman, T. 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxic assays. *J. Immunol. Methods.* 65:55–62.

42. Tada, H. 1986. An improved colorimetric assay for interleukin 2. *J. Immunol. Methods.* 93:157–163.

43. Kammer, G. M. 1988. The adenylate cyclase-cAMP protein kinase A pathway and regulation of the immune response. *Immunol. Today.* 9:222–229.

44. Bourne, H. R., L. M. Lichtenstein, K. L. Melmon, C. S. Henney, Y. Weinstein, and G. M. Shearer. 1974. Human lymphocyte metabolism: effect of cyclic and noncyclic nucleotides on stimulation by PHA. *Science (Wash. DC).* 184:19–22.

45. Johnson, K. W., B. H. Davis, and K. A. Smith. 1989. CAMP antagonizes interleukin-2 promoted T cell cycle progression at a discrete point in early G1. *Proc. Natl. Acad. Sci. USA.* 85:6072–6076.

46. Prince, H. E., V. Kermani-Arab, and J. L. Fahey. 1984. Depressed interleukin-2 receptor expression in acquired immune deficiency and lymphadenopathy syndrome. *J. Immunol.* 133:1313–1317.

47. Prince, H. E., and C. D. Czaplicki. 1988. In vitro activation of T lymphocytes from HIV-seropositive blood donors. II. Decreased mitogen-induced expression of interleukin 2 receptors by both CD4 and CD8 cell subsets. *Clin. Immunol. Immunopathol.* 48:132–139.