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Distinct modulatory effects of bryostatin 1 and staurosporine on the biosynthesis and expression of the HIV receptor protein (CD4) by T cells

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A family of structurally related macrocyclic lactones, bryostatins, have recently been shown to display several intriguing pharmacologic properties. Bryostatins are biosynthetic products of bryozoa phyllum of marine animals. To extend the analyses of the biological activities of these highly unusual biosynthetic animal products, we have examined the effect of bryostatin 1 (bryo-1) on the steady-state expression of the human immunodeficiency virus receptor, CD4, by normal peripheral blood T lymphocytes. Incubation of the cells with 5 nM bryo-1 caused a substantial loss of CD4 from the cell surface, as analyzed by flow cytometry using anti-CD4 monoclonal antibody. The modulation of CD4 expression by bryo-1 was not due to a cytotoxicity effect: in the culture conditions where it modulated CD4, bryo-1 also stimulated the expression of the interleukin 2 gene, as indicated by northern blot hybridization. In addition, incubation of the lymphocytes with nanomolar amounts of protein kinase C antagonist, staurosporine, resulted in the inhibition of the bryo-1–induced modulation of CD4 expression. The results of radioimmunoprecipitation analysis of detergent lysates of [^{35}S] methionine-labeled lymphocytes strongly suggest that bryo-1 inhibits the glycosylation and expression of CD4 in a manner similar to that of tunicamycin.

Introduction

The differentiation antigen, CD4, is predominantly expressed by the helper subpopulation of T lymphocytes. A substantial number of studies have previously shown that CD4 is the major receptor for human immunodeficiency virus (HIV)[1] (McDougal *et al.*, 1986; Smith *et al.*, 1987; Lifson *et al.*, 1988), the causative agent of the acquired immunodeficiency syndrome (AIDS) (Fauci, 1988). Infection of T cells with HIV is known to involve the binding of the viral envelope glycoprotein (gp120) to CD4 (McDougal *et al.*, 1986; Smith *et al.*, 1987; Lifson *et al.*, 1988). Thus anti-CD4 monoclonal antibodies and synthetic CD4 oligopeptides have recently been used to map the virus binding site on the CD4 molecule. These studies have suggested that the virus binds to a site located between amino acid residues 37–53 of CD4 (Jameson *et al.*, 1988). In experiments where recombinant CD4 gene was transfected and expressed in mouse cells, however, HIV was shown to bind to its receptor, but failed to establish an infection (Maddon *et al.*, 1986). This finding has led to the proposition that an additional cellular protein(s) of unknown identity may also play some role in the internalization or subsequent replication of the virus in T helper cells.

There is evidence that the interaction of CD4 with gp120 plays an additional role, which facilitates the fusion of the HIV particle with the cell membrane structure and endocytosis of the viral nucleocapsid. Furthermore, CD4 has been implicated in membrane fusion between HIV-infected T lymphocytes, a process that culminates in the formation of giant multinucleated cells (Maddon *et al.*, 1986; Lifson *et al.*, 1986a,b). Cell-to-cell fusion apparently plays a role in the transmission of virus between cells (Stein *et al.*, 1987). Thus, a model for CD4-mediated membrane fusion and endocytosis of the HIV nucleocapsid has been proposed (Fauci, 1988; Maddon *et al.*, 1986). In the study recently reported by Koga *et al.* (1990), it was observed

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[1] *Abbreviations:* bryo-1, bryostatin 1; IL-2, interleukin 2; PKC, protein kinase C; cAMP, adenosine 3′,5′-cyclic monophosphate; PMA, 4β-phorbol 12β-myristate 13α-acetate; PDD, 4α-phorbol 12,13-didecanoate; HIV, human immunodeficiency virus; AIDS, acquired immunodeficiency syndrome.

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that recombinant gp120 interacts with CD4 intracellularly to cause lysis of T cells.

Several classes of structurally distinct pharmacologic agents have recently been shown to abort the infection of T helper cells by HIV. Some of these reagents, such as aurintricarboxlic acid (Schols *et al.*, 1989), appear to inhibit viral infectivity by modulating the density of CD4 expressed on the cell surface. The observations suggest that intracellular events which regulate the synthesis or steady-state expression of CD4 could modify the infection of T4 cells by the virus. The study of biochemical pathways that control the synthesis and expression of CD4 would thus appear likely to aid in the design and development of other efficacious antagonists with the capability to alter the sensitivity of these lymphocytes to infection by HIV. In the present investigation, we have utilized a novel macrocyclic lactone, bryostatin 1 (bryo-1) (Figure 1), to analyze further the mechanism of regulation of CD4 expression by peripheral blood T cells.

Bryo-1 is one of many bryostatin congeners originally isolated from *Bugula neritina,* a bryozoa phyllum of marine animals (Pettit *et al.*, 1982, 1984). Computer-assisted molecular analysis suggests that bryo-1 is homologous with PMA and sn-1,2-diacylglycerol in the spatial distribution of some of the major PKC-activating epitopes (Kraft *et al.*, 1987). In contrast with tumor-promoting phorbol ester, however, bryo-1 is perhaps best known for its antineoplastic property. During the initial screening of bryostatins for biological activity, Pettit *et al.* (1982, 1984) reported that bryo-1 and bryo-4 extend the life span of tumor-bearing mice after *in vivo* administration. In addition, it was subsequently shown that bryo-1 also inhibits the PMA-induced carcinogenesis in mouse skin (Hennings *et al.*, 1987). These reports demonstrate important divergence in the biological activities of bryo-1 and phorbol ester and indicate the potential utility of PKC-activating bryostatins in studies where the tumor promoting property of phorbol ester may be undesirable. The study presented in this report indicates the potency of bryo-1 to modulate the expression of CD4 from the surface of T cells. Further evidence is presented that strongly implicates PKC in the mechanism of modulation of CD4 expression by the lactone.

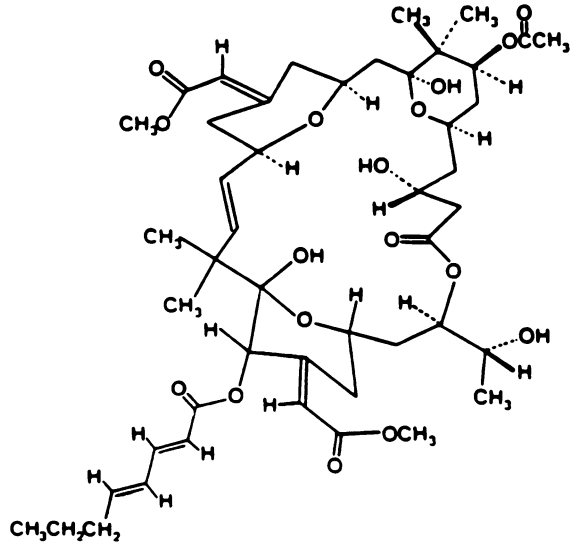

Figure 1. **Molecular structure of bryostatin 1 (Pettit *et al.*, 1982, 1984).**

Results

Modulation of CD4 expression by bryo-1

The effect of bryo-1 on the steady-state expression of CD4 was examined by means of flow cytometry using flurescein isothiocyanate-conjugated OKT4 monoclonal antibody. Incubation of the lymphocytes with 5 nM bryo-1 for 2 or 10 h caused a time-dependent loss of CD4 from the cell surface (Figure 2, left and middle). The number of $CD4^+$ cells declined to <3% by 10 h of incubation with bryo-1. CD4 was subsequently re-expressed by a significantly higher number of the lymphocytes after ~48 h of culture (Figure 2, right). We have confirmed the modulation of CD4 expression by bryo-1 by the use of additional anti-CD4 monoclonal antibodies: leu-3a and leu-3b (not shown). These antibodies as well as OKT4 have previously been reported to detect distinct epitopes of CD4. The consistency of the data derived from the use of these distinct anti-CD4 antibodies strongly suggests that bryo-1 promotes the loss of the entire CD4 molecular structure from the cell surface. Bryo-1 was also tested for its modulatory effect on two other T cell surface markers, CD2 and CD8, which are constitutively expressed by all, or by only the suppressor/cytotoxic T cells, respectively. We found no evidence that the reagent affects the expression of CD2 or CD8 in the same manner as observed for CD4. These additional findings show that bryo-1 down-regulates CD4 expression with a certain degree of specificity.

Distinct effects of bryo-1 and staurosporine on the expression of CD4

There are several reports indicating that bryo-1 competes with radiolabeled phorbol dibutyrate for binding to a common or closely related

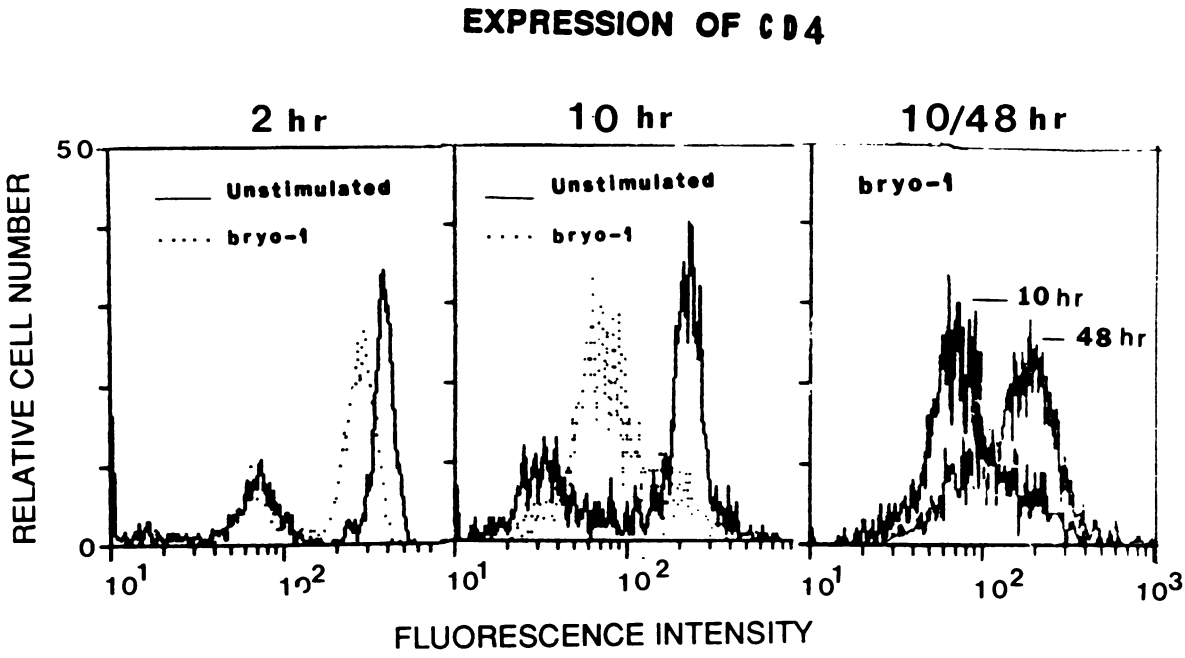

Figure 2. **Fluorescence histograms indicating time-dependent modulation of CD4 expression by bryo-1.** Unstimulated T cells or T cells incubated with bryo-1 were stained with fluorescein isothiocyanate-conjugated OKT4 monoclonal antibody. Two thousand live cells were gated and then analyzed for the expression of CD4 by means of a FacScan flow cytometer (Saksena *et al.,* 1988). The fluorescence histograms indicate the relative levels of CD4 expression by unstimulated T cells (——), and by T cells incubated with 5 nM bryo-1 (· · ·) for 2 h (left) or 10 h (middle). Also shown is the relative expression of CD4 by T cells incubated with bryo-1 for 10 h (——) or 48 hr (– – –).

receptors (Berkow and Kraft, 1985; Smith *et al.,* 1985; Kraft *et al.,* 1987). A large number of studies have further indicated that the phorbol ester receptor is the calcium- and phospholipid-activated cytosolic enzyme protein kinase C (PKC). Moreover, bryo-1 is also reported to mimic a number of the physiological effects of 4β-phorbol 12β-myristate 13α-acetate (PMA) through the activation of this enzyme (Berkow and Kraft, 1985; Smith *et al.,* 1985; Kraft *et al.,* 1987). To assess a potential role for PKC in the modulation of CD4 expression by bryo-1, we incubated T cells with 5 nM staurosporine, a relatively specific inhibitor for this enzyme. The cells were subsequently incubated with 5 nM bryo-1, washed, and then stained with fluorescein isothiocyanate-conjugated OKT4 monoclonal antibody. As analyzed by flow cytometry, a significantly larger number of cells treated with both bryo-1 and staurosporine expressed CD4 than those treated with bryo-1 alone (Figure 3, A–C). These results show that the ability of bryo-1 to modulate CD4 expression is antagonized by staurosporine. In a parallel set of experiments, we further explored the underlying mechanism of modulation of CD4 expression by the lactone. When compared by flow cytometric analysis, bryo-1 and PMA were nearly equipotent for the modulation of CD4 expression (Figure 3, B and E). In addition, the modulation of CD4 expression by these ligands was comparably sensitive to inhibition by the same dose (5 nM) of staurosporine (Figure 3, C and F). Contrary to these findings, however, when the lymphocytes were incubated with 4α-phorbol 12,13-didecanoate (PDD) alone or PDD in combination with staurosporine, there was no major change in the patterns of distribution of CD4 on the cell surface (Figure 3, G–I). It has been shown in previous reports that PDD neither activates PKC nor promotes PKC-dependent cellular responses, such as blast transformation of T cells (Isakov and Altman, 1987) or tumor development in mouse skin (Kikkawa *et al.,* 1985). These results (Figure 3) indicate that the modulation of CD4 expression by bryo-1 involves activation of the endogenous PKC. In addition, data obtained from the use of several other antagonists in additional experiments made it unlikely that other major protein kinases could also play a role in the modulation of CD4 by bryo-1. For instance, in identical culture conditions where staurosporine antagonized bryo-1 for the modulation of CD4 expression, we found that H-8 had essentially no effect (Figure 4). H-8 has previously been used in several

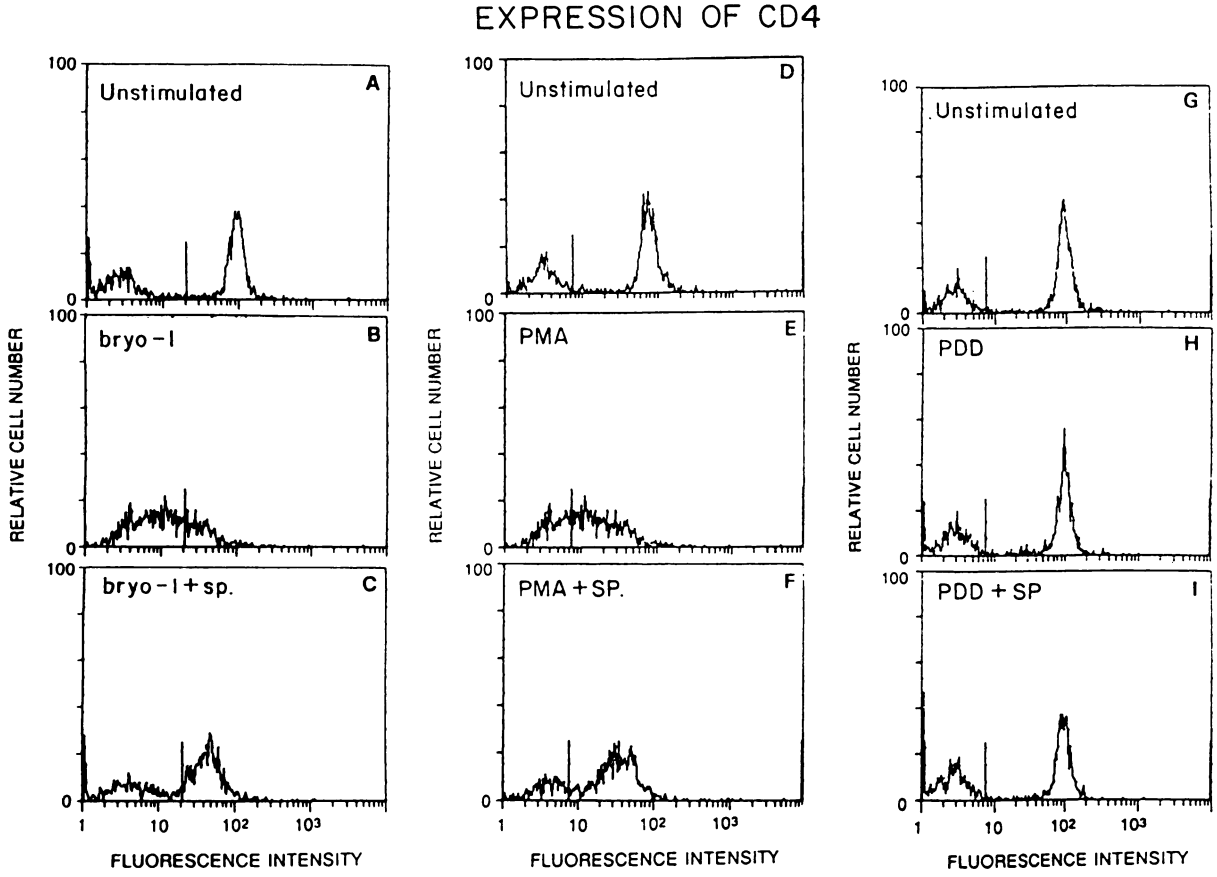

Figure 3. **Fluorescence histograms showing the effect of staurosporine on the modulation of CD4 expression by Bryo-1.** Expression of CD4 by unstimulated T cells (A), or by T cells incubated with 5 nM bryo-1 (B), 5 nM bryo-1 + 5 nM staurosporine [(SP) C]. Also shown are the effects of staurosporine on the modulation of CD4 by PMA (D–F) and the effects of 4α-phorbol 12,13-didecanoate (PDD) and staurosporine on the expression of CD4 (G–I).

studies for the specific inhibition of adenosine 3′,5′-cyclic monophosphate (c-AMP) dependent protein kinase.

There are several mechanisms that could account for the loss of CD4 from the surface of T lymphocytes after incubation with bryo-1. The reagent could reduce CD4 expression by promoting degradation or shedding of the antigen from the cell surface or by suppressing the biosynthesis or subsequent processing of the molecule or both. To examine some of these possibilities, we incubated T cells with bryo-1 for 24 h, at which point equal numbers of the cells were labeled with [^{35}S]methionine and lysed in immunoprecipitation buffer. The lysates were subjected to immunoprecipitation with OKT4 monoclonal antibody (Boto *et al.*, 1984). Autoradiogram of sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis of the immunoprecipitates from T cells cultured without bryo-1 shows that the monoclonal antibody detects two bands of [^{35}S]methionine-labeled molecules with sizes slightly less than standard BSA (Figure 5A, lane 1). The molecular weight estimates of the upper and lower bands are consistent with that of CD4. But the finding of potential interest concerns the distinctive effects of bryo-1 on the biosynthesis and relative accumulation of these proteins. Incubation of the cells with 10 nM bryo-1 results in the inhibition of the accumulation of the higher molecular weight band without a similar effect on the lower band (Figure 5A, lane 4). The shift in the size of the upper band induced by bryo-1 is comparable to that observed in the immunoprecipitate of T cells cultured in 5 μM tunicamycin for 24 h (Figure 5A, lane 5). We suggest that the lower band observed in the immunoprecipitate of OKT4 monoclonal antibody is a precursor of CD4 on its way to glycosylation.

The modulatory effect of bryo-1 on the biosynthesis or processing of CD4 appeared to be specific for this antigen. When the same detergent lysates of bryo-1–modulated T cells were

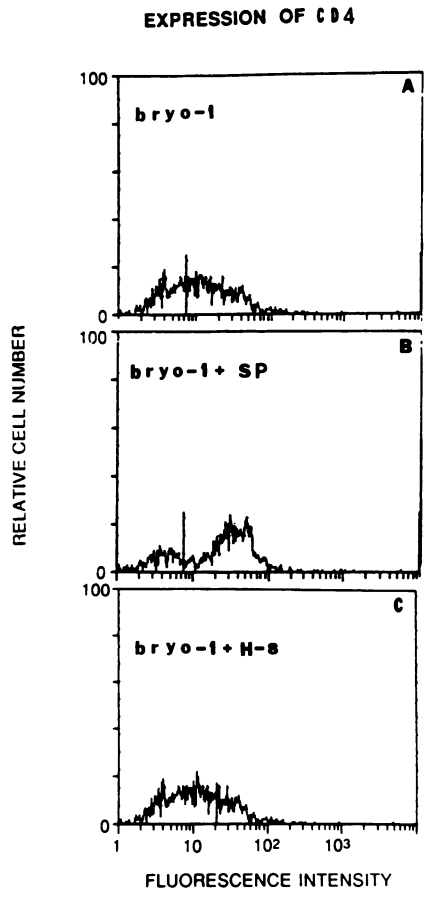

***Figure 4.* Effect of H-8 on the modulation of CD4 expression by bryo-1.** Expression of CD4 by T cells incubated with 5 nM bryo-1 (A), 5 nM bryo-1 + 5 nM staurosporine [(SP) B], and 5 nM bryo-1 + 7 μM H-8 (C).

subjected to a second cycle of immunoprecipitation with the pan-T cell–specific anti-CD6 monoclonal antibody, a totally different set of results were obtained. Neither bryo-1 (Figure 5B, lanes 2–4) nor tunicamycin (lane 5) had a noticeable effect on the levels of the accumulation of CD6 despite the fact that this antigen is also glycosylated. The difference in the effects of the lactone on the processing of CD4 and CD6 probably indicates that the antigens are glycosylated at different sites or by separate mechanisms in the cell.

The apparent inhibition of the processing of CD4 and the loss of the antigen from the surface of T cells after incubation with bryo-1 are consistent with several interpretations. Since only viable cells were gated for flow cytometry (Saksena *et al.,* 1988), these tests could not rule out the possibility that bryo-1 was selectively cytotoxic for $CD4^+$ T lymphocytes in culture. Alternatively, CD4 expression could be modulated as a part of the mechanism by which bryo-1 may promote the immune-associated response of T4 cells. A mechanism involving the modulation of CD4 as well as TCR/CD3 expression has been previously proposed for the induction of the interleukin 2 (IL-2) gene and other immune-associated response by other PKC-dependent mitogens. Moreover the secretion of IL-2 is known to be an exclusive function of T_4 cells. To explore these possibilities, we tested whether bryo-1 would affect the expression of the IL-2 gene in the culture conditions where it modulates CD4 biosynthesis and expression.

Induction of IL-2 gene expression by bryo-1

When added to T cells in the presence of calcium ionophore A23187, bryo-1 generates a strong signal that triggers transient expression of the IL-2 gene within 6 h of culture, as revealed by northern blot hybridization (Figure 6A). The addition of bryo-1 alone to the cultures was not sufficient to induce the gene. Other findings made it unlikely that the ionophore contributed to the induction of the lymphokine gene by reversing the modulatory effect of bryo-1 on CD4 expression. We found that A23187 had no effect on the expression of CD4, nor did it alter the modulation of CD4 expression by bryo-1. The results of the IL-2 gene transcription assay make it unlikely that the inhibition of the biosynthesis and expression of CD4 by T cells were due to a cytotoxicity effect of bryo-1. On the contrary, the observations raise speculation that the level of CD4 expression may in some manner affect de novo transcription of the IL-2 gene. This possibility can not be overlooked entirely, particularly in view of the fact that the PKC-specific antagonist, staurosporine, inhibits the bryo-1–induced modulation of CD4 as well as induction of the IL-2 gene (Fig. 6B) with comparable potency. Indeed several studies have previously shown that the expression of CD4 and other immunoregulatory surface derminants, such as Ti/CD3 complex, can be radically reduced by PKC-activating agents (Hoxie *et al.,* 1986; Blue *et al.,* 1989). These findings lead the respective investigators to propose that the modulation of CD4 expression may be an essential element of signal transduction for the activation of T cells. Nevertheless, evidence for a direct cause-and-effect relationship between the alteration in the surface density of this determinant and signal transduction has not been forthcoming.

Discussion

This study has shown that a highly potent nontumorigenic PKC agonist, bryo-1, and PKC an-

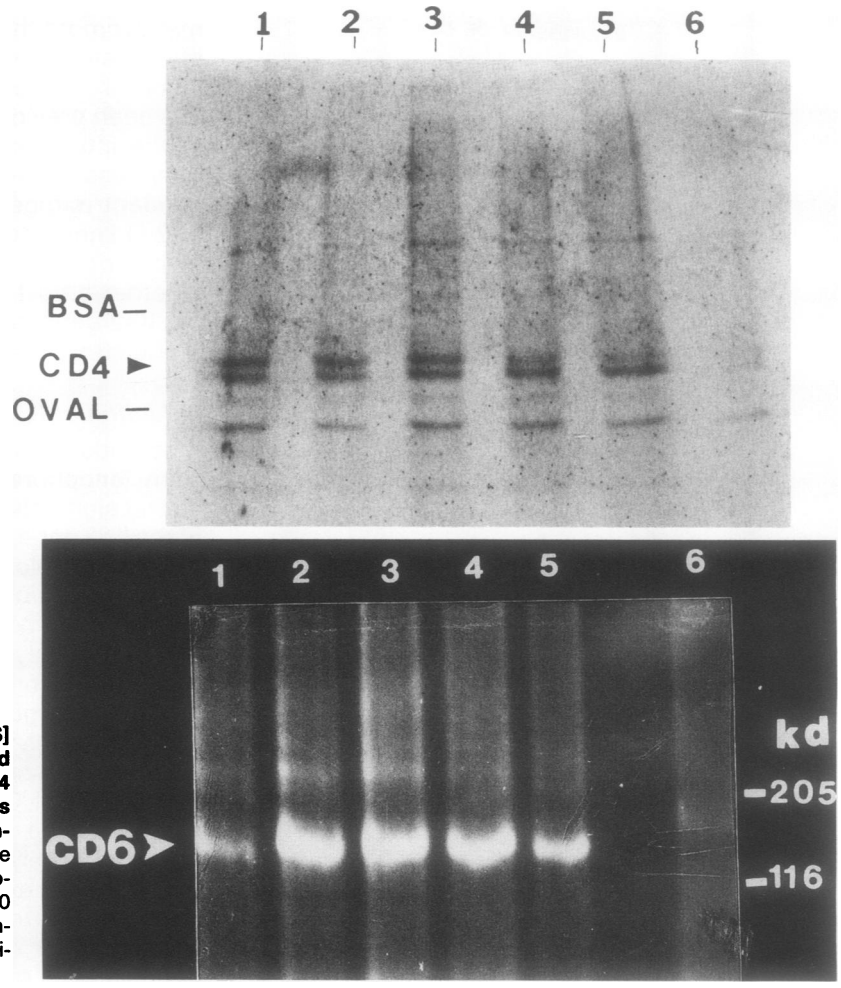

***Figure 5.* Autoradiogram of [^{35}S] methionine-labeled CD4 as detected by immunoprecipitation with OKT4 monoclonal antibody (A), and CD6 as detected by anti-CD6 monoclonal antibody (B).** A and B: normal T cells (lane 1); T cells incubated with 0.1 nM bryo-1 (lane 2); 1 nM bryo-1 (lane 3); and 10 nM bryo-1 (lane 4); and 5 μM tunicamycin (lane 5); lysate of T cells precipitated with nonimmune IgG (lane 6).

tagonist, staurosporine, can be utilized individually or in appropriate combination to set limits on the density of CD4 expressed on the surface of T helper cells. Both ligands were shown to control the expression of CD4 when added to the cells at nanomolar range of concentrations.

The results of radioimmunoprecipitation analysis of detergent lysates of T cells suggested that bryo-1 inhibits the glycosylation of CD4 and may thus modulate the expression of the antigen via this mechanism. In this regard the lactone apparently mimics the effect of tunicamycin, a reagent previously reported to inhibit the glycosylation as well as expression of CD4 (Konig *et al.,* 1986). Other studies have previously shown that the expression of CD4 is modulated by tumor-promoting phorbol esters (Hoxie *et al.,* 1986; Blue *et al.,* 1989). In these instances, however, the agents were reported to alter CD4 expression through the internalization of the molecule. These earlier observations together with our own data suggest that bryo-1 modulates CD4 expression by a mechanism distinct from that of tumor promoters. The finding potentially extends the scope for the application of the lactone as probe for cellular signals unrelated to neoplastic growth.

The results of flow cytometric analysis had indicated that staurosporine suppresses the modulatory effect of bryo-1 on the expression of CD4 and upregulates the density of the antigen found on the cell surface. We were therefore surprised to note that the reagent had essentially no effect on the ability of bryo-1 to inhibit the biosynthesis of the antigen. It is conceivable that staurosporine upregulates CD4 expression in bryo-1–incubated cells via a PKC-dependent mechanism not associated with the biosynthesis or glycosylation of the molecule. One possible mode of action consistent with this scenario is that the reagent inhibits the turnover rate or degradation of CD4. This subject is under investigation.

The ability to modulate the presentation of the HIV receptor, CD4, on the cell surface would appear to provide a novel strategy that may be

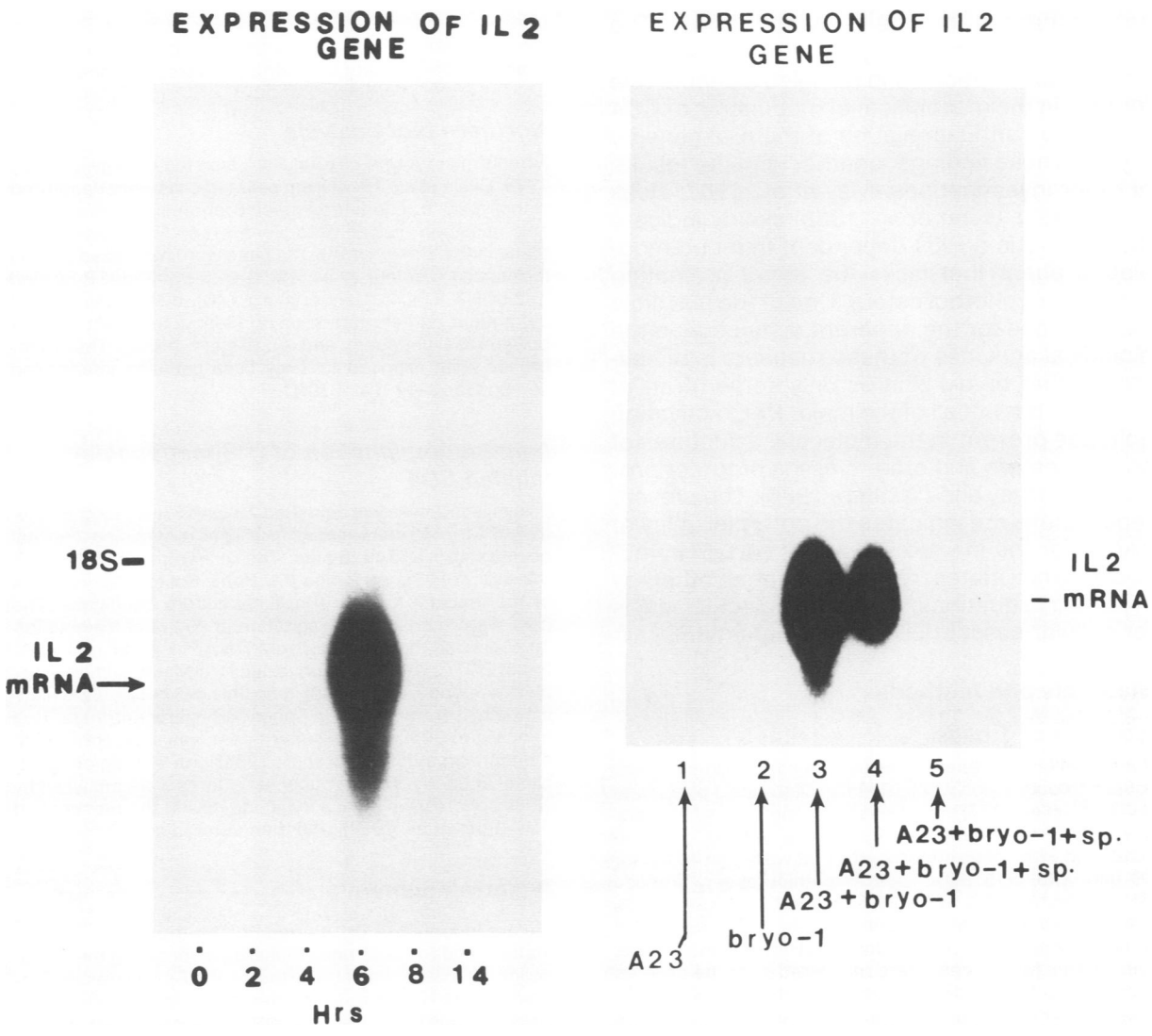

Figure 6. **Expression of IL-2 gene.** (A) Autoradiogram of northern blot indicating transient accumulation of the IL-2 mRNA induced by incubation of T cells with bryo-1 + A23187. (B) Effect of staurosporine on steady-state accumulation of IL-2 mRNA in T cells incubated with bryo-1 + A23187. Levels of the IL-2 mRNA in T cells incubated with A23187 (lane 1), bryo-1 (lane 2), A23187 + bryo-1 (lane 3), A23187 + bryo-1 + 5 nM staurosporine (lane 4), and A23187 + bryo-1 + 15 nM staurosporine (lane 5). Bryo-1 and A23187 were used at 5 nM and 0.3 μM, respectively, in all cases.

useful for the elucidation of the mechanism of recognition and infection of T helper cells by the virus and possibly for the design of novel antagonists with the capability to modify the susceptibility of these lymphocytes to the infection. The selective loss of CD4 from the cell surface and depletion of T helper cells have often been associated with the acute phase of HIV infection in vitro (Fauci, 1988; Lifson *et al.*, 1986b) and immunodeficiency of T4 cells in subjects with AIDS (Fauci, 1988). Nevertheless, the evidence presented in this report appears to support the paradoxical conclusion that the biosynthesis and expression of CD4 can be dramatically altered in vitro without an apparent impairment in the helper function of T4 lymphocytes, at least as quantified by the ability of the cells to express the IL-2 gene. These observations therefore indicate the feasibility of utilizing bryo-1 and staurosporine to probe and modulate the infection of T4 cells by HIV.

May *et al.* (1987) and Hess *et al.* (1988) have previously shown that bryo-1 promotes the development of murine bone marrow progenitor stem cells and secretion of IL-2 by T lymphocytes, respectively. Another report (Trenn *et al.*, 1988) has also indicated that bryo-1 greatly enhances the IL-2–dependent cytolytic activity of

antigen-primed murine T lymphocytes. The present study has partly confirmed these important observations and revealed a central role for PKC in the mechanism of modulation of CD4 expression and stimulation of the IL-2 gene by bryo-1. These findings together with the results of other investigations (May *et al.*, 1987; Hess *et al.*, 1988; Trenn *et al.*, 1988) clearly indicate that bryo-1 is a PKC-dependent immuno-modulating agent that lacks the tumor-promoting activities of phorbol esters. One of the plausible explanations for the apparent distinction in the biological activities of these reagents is the evidence that bryo-1 shares only some (Kraft *et al.*, 1987) but not all of the major PKC-activating epitopes present in the molecular structures of phorbol esters and other classes of tumor promoters (Jeffrey and Liskamp, 1986). The present report therefore indicates the potential utility of bryo-1 for the in vitro studies of certain immunodeficiency states, restorative immunotherapy in certain situations, and the molecular basis for cellular susceptibility to HIV infection.

Materials and methods

Isolation of T cells

Peripheral blood mononuclear cells of normal donors were passed through nylon wool column in Dulbecco's phosphate buffered saline (PBS) containing Ca^{2+}, Mg^{2+}, and 10% fetal bovine serum. Nonadherent cells were washed and then incubated with sheep erythrocytes that were pretreated for 15 min with 0.15 M aminoethylisothiouronium bromide (Sigma Chemical Co., St. Louis, MO) (pH 8.0). Rosetted cells were separated from nonrosetting cells by centrifugation through lymphocyte separation medium (Liton Bionics, Kensington, MD). T cells were recovered from the pelleted rosettes upon lysis of erythrocytes with 5 ml of 58 mM tris(hydroxymethyl)aminomethane (Tris)-ammonium chloride buffer (pH 7.2) for 5–8 min. At least 97% of the leukocytes expressed the sheep erythrocyte receptor, as revealed by flow cytometric analysis using OKT11 (anti-CD2) monoclonal antibody.

Activation of T cells

T cells were resuspended at a density of 2×10^6/ml in culture medium: RPMI 1640 (Gibco, Grand Island, NY) + 10% fetal bovine serum + 100 μg/ml streptomycin + 100 U/ml penicillin. Aliquots of the cells were preincubated in staurosporine (Kyowa Hakko, Inc., NY) for at least 2 h in Falcon tissue culture flasks. The cells were then incubated with bryo-1 (May *et al.*, 1987; Hess *et al.*, 1988) alone or bryo-1 plus A23187 (Sigma Chemical Co), as required. The cultures were incubated at 37°C in 5% CO_2 and 95% humidity.

Flow cytometry

3×10^5 cultured cells were washed and then incubated with excess amounts (0.2 μg) of fluorescein isothiocyanate-conjugated OKT4 (anti-CD4) monoclonal antibody (Ortho Diagnostics, Rareton, NJ) for 30 min at 4°C. The cells were washed and then resuspended in 1 ml of chilled PBS containing 1% fetal bovine serum. Flow cytometric analysis was performed (Saksena *et al.*, 1988) using a FACScan flow cytometer (Becton Dickinson Immunocytometry Systems, Mountain View, CA). Histograms demonstrating relative fluorescence intensity of $CD4^+$ cells were developed.

Northern blot analysis

Fifteen micrograms of total RNA isolated (Chirgwin *et al.*, 1979; Gilsin *et al.*, 1974) from pelleted cells were denatured with glyoxal, fractionated by electrophoresis in 1% agarose gels and then blotted onto Genescreen Plus nylon membrane (NEN, Boston, MA). The blots were hybridized in 50% formamide (LeMeur *et al.*, 1981) with (α-^{32}P)dATP-labeled IL-2 cDNA (Oncor, Gaithersburg, MD). cDNA was labeled by random primer method using Klenow fragment of DNA polymerase (Feinberg and Vogelstein, 1983). The membranes were exposed to X-ray films between intensifying screens for 8–12 h at −70°C.

Immunoprecipitation of [^{35}S]methionine-labeled CD4

Five million T cells were washed 2× with 5 ml of methionine-free RPMI 1640 culture medium (Sigma) containing 2% fetal bovine serum and antibiotics. The cells were incubated with 0.2 mCi of [^{35}S]methionine (Du Pont, Boston, MA) in 2 ml of the medium for 2 h in optimal culture conditions. The cells were then lysed by agitation in 250 μl of ice-cold immunoprecipitation buffer (50 mM Tris, pH 7.4; 150 mM NaCl; 5 mM EDTA; 0.02% sodium azide; 1 mM phenylmethylsulphonyl fluoride; 1% NP-40). Insoluble cell debris were eliminated by centrifugation in Eppendorf microcentrifuge. Two hundred to 300 μl of the supernatant was subjected to immunoprecipitation (Boto *et al.*, 1984) with 2–5 mg of monoclonal antibody. The immunoprecipitates were analyzed by electrophoresis in 10% acrylamide gels in the presence of SDS (Boto *et al.*, 1984), and then autoradiographed.

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