Calcium-mediated inhibition of phorbol ester and Tax trans-activation of the human T cell leukaemia virus type 1

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Introduction

Human T cell leukaemia virus type 1 (HTLV-1) infection is characterized by a long asymptomatic period in infected individuals. In a relatively low percentage of HTLV-1-seropositive individuals, infection is associated with adult T cell leukaemia/lymphoma (ATL) (Hinuma et al., 1981; Poiesz et al., 1980; Yoshida et al., 1982) and the inflammatory/degenerative neurological disorders tropical spastic paraparesis (TSP) and HTLV-1-associated myelopathy (HAM) (Osame et al., 1986; Gessain et al., 1985). ATL is a malignancy of helper T lymphocytes whereas HAM/TSP is a myelopathy which shares some of the characteristics of multiple sclerosis. The peripheral blood mononuclear cells of infected individuals undergo spontaneous proliferation in vitro (Itoyama et al., 1988; Jacobson et al., 1988; Tendler et al., 1990) which may result from the activation of expression of interleukin 2 (IL-2) and its receptor (IL-2R) by HTLV-1 (Siekevitz et al., 1987a). The alteration of normal cellular function by HTLV-1 has been proposed to be associated with the expression of viral proteins such as the trans-activator Tax which can induce IL-2R expression (Tendler et al., 1990).

Positive regulators of HTLV-1 LTR-directed gene expression have been defined and include 12-O-tetradecanoylphorbol-13-acetate (TPA) (Radonovich & Jeang, 1989) and Tax (Chen et al., 1985; Radonovich & Jeang, 1989; Sodroski et al., 1985; Varmus, 1988). The response of the LTR to TPA-mediated T cell activation is determined by a 60 bp sequence which overlaps one Tax-responsive element (Radonovich & Jeang, 1989). TPA directly activates protein kinase C (PKC) which is one of several pathways required during T cell activation. The trans-activation of HTLV-1 expression by Tax is mediated via Tax-responsive elements reiterated three times within the LTR (Chen et al., 1985; Sodroski et al., 1985; Varmus, 1988). Transcriptional regulation of cellular and other viral promoters by Tax has been reported (Albrecht et al., 1992; Arya, 1988; Fox et al., 1989; Fuji et al., 1988; Leung & Nabel, 1988; Lindholm et al., 1992; Lilienbaum et al., 1990; Siekevitz et al., 1987b). The pathways by which Tax and TPA transactivate LTR-mediated gene expression are independent, demonstrated by the synergistic response of the LTR when both agents are used together (Radonovich & Jeang, 1989). Hence, upregulation of HTLV-1 expression has been linked to T cell activation events mediated via PKC expression. However, despite the constitutive expression of IL-2R (Depper et al., 1984; Popovic et al., 1983; Yodoi et al., 1983) and in some cases the production of IL-2 (Depper et al., 1984; Gootenberg et al., 1981; Hattori et al., 1981) by circulating HTLV-1-infected T lymphocytes in vivo, HTLV-1 gene products are expressed at low levels (Franchini et al., 1984; Hoshino et al., 1983; Sugamura et al., 1984). Viral regulatory protein transcripts have been detected using reverse transcriptase PCR in lymphocytes of HTLV-1-infected patients, but expression is low and occurs in only a small number of cells (Gessain et al., 1991;
Kinoshiba et al., 1989). This suggests that a negative regulatory mechanism may play an important role in maintaining HTLV-1 in a latent-like state, allowing persistent asymptomatic infection in the majority of infected cells in seropositive individuals.

In HeLa cells we have observed previously that although the LTR is responsive to TPA, this response is significantly reduced by the calcium ionophore ionomycin (Copeland et al., 1994). Ionomycin enhances both the release of Ca\(^{2+}\) from intracellular stores and the influx of Ca\(^{2+}\) from the extracellular environment (Clevers et al., 1988; Gardner, 1989; Gelfand et al., 1987). Thus a pathway that enhances the free Ca\(^{2+}\) concentration within the cell may create conditions unfavourable for virus expression. A role for Ca\(^{2+}\) during HTLV-1 infection has been inferred by studies of both HTLV-1 expression and Tax-mediated trans-activation of cellular genes. In HTLV-1-producing MT-2 T cells, increases in intracellular free Ca\(^{2+}\) have been shown to support the inhibition of cellular proliferation mediated by the parathyroid hormone-related protein (PTHrP) (McCaulay et al., 1992). This observation is particularly interesting since the expression of PTHrP can be positively regulated by Tax (Ejima et al., 1993). Tax activity has been previously shown to be insensitive to some Ca\(^{2+}\)-dependent pathways. Although activation of the granulocyte-macrophage colony stimulating factor (GM-CSF) promoter by phorbol esters plus calcium ionophore is sensitive to cyclosporin A (CsA), Tax can overcome this transcriptional block (Koyano-Nakagawa et al., 1993). Increases in intracellular Ca\(^{2+}\) also mediate phosphorylation of the Ets-1 nuclear protein (Fisher et al., 1991). Ets-1 acts as a transcriptional activator of the HTLV-1 LTR (Bosselut et al., 1990) via the Ets-1 binding site which is required for inducible HTLV-1 enhancer function (Clark et al., 1993). Phosphorylation of Ets-1 may result in a decrease in the level of association of the protein with the LTR and have a negative effect on virus expression.

In this study we have used Jurkat T cells as a model for dissecting the Ca\(^{2+}\)-dependent events which are responsible for the inhibition of HTLV-1 expression by ionomycin. Using various Ca\(^{2+}\) pathway inhibitors we have studied Ca\(^{2+}\)-dependent events in this T cell line containing a stably integrated HTLV-1 LTR-regulated reporter gene. We report on the role of Ca\(^{2+}\)-dependent events in the regulation of HTLV-1 expression and discuss the implications of these findings for a possible mechanism of HTLV-1 persistence and latency.

**Methods**

**Construction of vectors and stable transfections.** The vector pHTLV-1 LTR–lacZ-neo has been described previously (Copeland et al., 1993). The LTR region of this vector spans –322 to +332 bp relative to the cap site. pRSVtax is the HTLV-1 equivalent of the vector pBLVtax (Dense & Martarano, 1990). The vector pHTLV-1 LTR–lacZ-neo was introduced into human Jurkat cells by electroporation. Jurkat cells (2 x 10⁶) were suspended in 250 μl RPMI culture medium supplemented with 20% fetal calf serum (FCS). The cells were mixed with 10 μg of plasmid DNA and transferred to a 0.4 cm gap electroporation cuvette and electroporated (250 V, 960 μF) using a Gene Pulser with capacitance extender (Bio-Rad). Cells were cultured for 2 days in RPMI supplemented with 20% FCS, 100 U/ml penicillin and 100 μg/ml streptomycin before selection in 1 mg/ml G418 (Gibco-BRL). Cells were later cultured in Iscove’s modification of Dulbecco’s medium for cloning from single cells by limiting dilution.

**Stimulation experiments.** pHTLV-1 LTR–lacZ-expressing Jurkat cells were transfected with 10 μg pRSVtax using a modified DEAE-dextran transfection procedure (Gruters et al., 1991). Following 40 h of culture, transfected and untransfected cells were split for treatment with ionomycin (2 μM; Sigma), TPA (10 ng/ml; Sigma) or TPA plus ionomycin, with or without the inhibitors EGTA (2 mM), 1,2-bis(2aminophenoxy)ethane-N,N,N’,N’-tetraacetic acid (BAPTA/AM 5 μM; Sigma) or CsA (1 μg/ml; Sandoz). Culture medium was supplemented with 1 mM-Ca\(^{2+}\). To chelate extracellular Ca\(^{2+}\), cells were treated with EGTA for 5 min prior to the addition of TPA or ionomycin. For this treatment group EGTA was kept in the culture medium during the entire stimulation period. In the case of intracellular Ca\(^{2+}\) chelation, cells were incubated in 5 μM-BAPTA/AM for 1 h and the cells were then washed and stimulated in the absence of BAPTA/AM. CsA was added to cells 10 min before the addition of TPA or ionomycin. Stimulation with TPA or ionomycin was for 18 h duration.

**Measurement of β-galactosidase.** β-Galactosidase (lacZ) activity was measured from the fluorescence produced following cleavage of the β-galactoside bond of 4-methylumbelliferyl-β-D-galactoside (MUG; Molecular Probes). Cells were washed with Z buffer (60 mM-Na₂HPO₄, 40 mM-NaH₂PO₄, 10 mM-KCl, 1 mM-MgSO₄, pH 7.0) and lysed in Z buffer containing 0.1% Triton X-100. For fluorimeter measurements, 20 μl of 50 mM-MUG was combined with 2 ml PBS and the lysate of 2 x 10⁶ cells (100 μl) in a quartz cuvette. Fluorescence at 376 nm was measured for 1 min using 350 nm excitation with a luminescence spectrometer (LS50, Perkin Elmer). The rate of increase in fluorescence was calculated using linear regression analysis. Differences in lacZ expression between treatment groups were evaluated using Student’s t-test.

**Measurement of HTLV-1 expression.** HTLV-1 expression was detected as p24 antigen production using an ELISA kit (HTLV-1, II Antigen Assay; Coulter). Equal numbers of MT-2 cells were cultured in the presence of different levels of ionomycin, CsA or ionomycin plus CsA for 72 h. The supernatants were then used to measure p24 antigen, according to the manufacturer’s specifications. At the same time cell viability was determined using trypan blue exclusion to ensure that observed changes in virus production were not due to toxic effects of the reagents. Variation in p24 production between different treatment groups was evaluated using Student’s t-test.

**Measurement of intracellular Ca\(^{2+}\).** Cells were prepared for intracellular Ca\(^{2+}\) measurement according to Vandenbergh & Ceuppens (1990) with minor modifications. Briefly, cells (10⁷/ml) were incubated for 20 min under subdued light at room temperature in Hanks’ balanced salt solution (HBSS) containing 20% Pluronic F-127. Cells were washed with Z buffer (60 mM-Na₂HPO₄, 40 mM-NaH₂PO₄, 10 mM-KCl, 1 mM-MgSO₄, pH 7.0) and lysed in Z buffer containing 0.1% Triton X-100. For fluorimeter measurements, 20 μl of 50 mM-MUG was combined with 2 ml PBS and the lysate of 2 x 10⁶ cells (100 μl) in a quartz cuvette. Fluorescence at 376 nm was measured for 1 min using 350 nm excitation with a luminescence spectrometer (LS50, Perkin Elmer). The rate of increase in fluorescence was calculated using linear regression analysis. Differences in lacZ expression between treatment groups were evaluated using Student’s t-test.
cells for 1 h and then removed prior to staining with Fluo-3/AM. In the case of CsA and EGTA, these were added to the cells 10 min prior to staining with Fluo-3/AM and were present in the solutions used in all subsequent steps. The cells were incubated at 37 °C for 10 min before measurement. Each treatment group was subdivided into four groups which received either no additions or ionomycin (2, 5 and 10 μM). Fluo-3/AM fluorescence (green) was measured at selected time points following the addition of ionomycin, using a FACScan flow cytometer (Becton Dickinson). Excitation was from an argon laser at 488 nm. Emission at 530 nm was measured on a logarithmic scale. During the sampling period the cells were maintained at 37 °C.

Results

Ionomycin is inhibitory to activation of the HTLV-1 LTR by TPA and Tax

To investigate the requirements for cellular free Ca²⁺ in trans-activation of the HTLV-1 LTR, a Jurkat T cell clone containing a stably integrated HTLV-1 LTR–lacZ gene was used. LTR-directed lacZ expression was measured by fluorimetry of cell lysates stained with the fluorogenic substrate MUG and is reported as the fold increase in lacZ expression obtained in stimulated cells with respect to untreated cells. We have previously reported on the inhibitory effect of the Ca²⁺ ionophore ionomycin on the response of the HTLV-1 LTR to TPA in stably transfected HeLa cells (Copeland et al., 1993). We report here that this inhibition is also found in the Jurkat T cell line. Fig. 1 shows the effect of ionomycin (2 μM) on basal (uninduced) LTR activity and on activation mediated by TPA, Tax or the combined treatment of Tax plus TPA. The concentration of ionomycin used in these experiments is similar to that employed in other studies of gene expression during T cell activation (Fiering et al., 1990; Gruters et al., 1991; Mattila et al., 1990; Siekevitz et al., 1987a; Ullman et al., 1993). In all cases ionomycin reduced LTR-directed gene expression by 20 to 25%. For all treatment groups the level of lacZ expression in the presence of ionomycin was

![Fig. 1. Inhibition of LTR-mediated lacZ expression by ionomycin.](image)

Fig. 1. Inhibition of LTR-mediated lacZ expression by ionomycin. pHTLV-1-expressing Jurkat cells were treated as indicated in the presence or absence of 2 μM-ionomycin. Fluorescence was measured in MUG-stained lysates as described in Methods. The values shown are the means and standard deviations obtained from three independent experiments.

![Fig. 2. Ionomycin-induced Ca²⁺ flux in Jurkat cells treated with pathway inhibitors.](image)

Fig. 2. Ionomycin-induced Ca²⁺ flux in Jurkat cells treated with pathway inhibitors. Prior to Fluo-3/AM staining the cells were either untreated (a) or treated with (b) 5 μM-BAPTA/AM, or (c) 2 mM-EGTA. To measure Ca²⁺ mobilization, stained cells were treated with ionomycin at 2 μM (□), 5 μM (○) or 10 μM (△). Ionomycin was added to the cells at 0 min. Each point represents the percentage of the cell population positive for Fluo-3/AM fluorescence. A representative of three independently performed experiments is shown.
The effect of CsA on intracellular Ca\textsuperscript{2+} mobilization

In the case of cells treated with the immunosuppressive drug CsA (Fig. 3), the bimodal release of Ca\textsuperscript{2+} was similar to that observed in the absence of CsA (Fig. 2a). CsA inhibits Ca\textsuperscript{2+}-dependent signal transduction events essential for the expression of genes required during T cell activation (Emmel et al., 1989; Flanagan et al., 1991; Mattila et al., 1990; Randak et al., 1990). The effectiveness of CsA was further tested using a Jurkat cell line expressing a trimeric NF-AT element of the IL-2 gene promoter linked to the lacZ reporter gene (Fiering et al., 1990). The response of the NF-AT element to TPA plus ionomycin stimulation was efficiently blocked by CsA at 1 \mu g/ml (data not shown) as has been described previously (Flanagan et al., 1991; McCaffrey et al., 1993; Sickevitz et al., 1987a).

**Ionomycin-mediated inhibition is recovered by CsA**

Regulation of HTLV-1 expression was then investigated in cells treated with Ca\textsuperscript{2+} inhibitors in an attempt to identify the mechanism of inhibition by ionomycin. Cells were treated with EGTA (2 mM) to chelate extracellular Ca\textsuperscript{2+}, BAPTA/AM (5 \mu M) to chelate intracellular Ca\textsuperscript{2+} or with CsA (1 \mu g/ml) to inhibit Ca\textsuperscript{2+}-sensitive T cell signal transduction pathways. As experimental controls, basal LTR activity was tested in cells treated with Ca\textsuperscript{2+} inhibitors in the absence or presence of ionomycin (Fig. 4a). Basal lacZ expression in the absence of ionomycin was unaffected by chelation of intracellular Ca\textsuperscript{2+} by BAPTA/AM but showed a dependence upon extracellular Ca\textsuperscript{2+} availability, demonstrated by reduced lacZ expression in the presence of EGTA (Fig. 4a; untreated). CsA was able to enhance basal LTR activity slightly. Both the decreased lacZ expression in EGTA-treated cells and the increased expression in the presence of CsA were found to be significantly different from basal expression (P < 0.01). In cells treated with ionomycin (Fig. 4a; ionomycin) basal LTR expression was mildly decreased but this inhibition was not observed in the presence of ionomycin plus CsA. In fact, ionomycin and CsA acted synergistically resulting in a significant increase in lacZ expression over basal levels (P < 0.01).

Regulation of expression by TPA-mediated PKC activation was shown to be dependent upon intracellular Ca\textsuperscript{2+} levels (P < 0.01) because BAPTA/AM treatment reduced the lacZ activity to near basal levels in TPA-treated cells (Fig. 4b; TPA). TPA-mediated expression was not affected by treatment with CsA. Ionomycin suppressed TPA-mediated responses (Fig. 4b; TPA+ ionomycin) and this decrease in gene expression was not alleviated when intracellular or extracellular Ca\textsuperscript{2+} was chelated by BAPTA/AM and EGTA, respectively. However, the suppression of TPA responses induced by ionomycin was overcome in the presence of CsA and was restored to levels significantly higher than those observed in the absence of ionomycin (P < 0.01).

The requirements for Ca\textsuperscript{2+} in Tax-mediated transactivation of LTR-directed HTLV-1 expression were investigated following transient transfection of the pHTLV-1-LTR-lacZ-expressing cells with pRStax (Fig. 4c). The response of the LTR to Tax was not adversely affected by chelation of intracellular Ca\textsuperscript{2+} by...
Inhibition of HTLV-1 by ionomycin

Fig. 4. Investigation of ionomycin-induced inhibition using Ca\(^{2+}\) pathway inhibitors. Fluorescence was measured in lysates of HTLV-1 LTR–lacZ cells. Cells were stimulated in the presence or absence of 2 μM-ionomycin with (a) culture medium alone; (b) TPA, 10 ng/ml; (c) TPA + ionomycin; (d) Tax + ionomycin. Untreated cells served as controls. TPA, 10 ng/ml; Tax, 1 lag/ml; ionomycin, 2 μM; CsA, 1 μg/ml; BAPTA/AM, 5 μM; EGTA, 2 mM; and trypan blue exclusion was used to determine viable cell number.

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Table 1. Influence of ionomycin and CsA on HTLV-1 production in MT-2 cells

<table>
<thead>
<tr>
<th>Ionomycin (μM)</th>
<th>CsA (1 μg/ml) per 10(^6) viable cells*</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100 ± 5.3</td>
</tr>
<tr>
<td>2</td>
<td>101 ± 7.9</td>
</tr>
<tr>
<td>5</td>
<td>90 ± 4.9</td>
</tr>
<tr>
<td>10</td>
<td>57 ± 3.5</td>
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<tr>
<td>0</td>
<td>71 ± 5.6</td>
</tr>
<tr>
<td>2</td>
<td>67 ± 5.5</td>
</tr>
<tr>
<td>5</td>
<td>64 ± 3.6</td>
</tr>
<tr>
<td>10</td>
<td>72 ± 4.5</td>
</tr>
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</table>

* Viruses were produced using an HTLV-I/II p24 antigen capture kit. Viable cell number was calculated using trypan blue exclusion prior to p24 measurement. The results are the means and standard deviations of three independent experiments.

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BAPTA/AM in contrast to TPA-induced expression (Fig. 4c; Tax). Ionomycin treatment reduced the response of the LTR to Tax-mediated trans-activation and this suppressive effect was alleviated significantly by chelation of intracellular Ca\(^{2+}\) by BAPTA/AM (P < 0.01) and to a greater degree by both EGTA and CsA treatment (Fig. 4c; Tax + ionomycin). A synergistic effect of Tax plus TPA on HTLV-1 expression was also demonstrated in the LTR–lacZ cells (Fig. 4d) in agreement with previous reports (Radonovich & Jeang, 1989). The chelation of intracellular and extracellular Ca\(^{2+}\) mildly reduced the synergistic response of the LTR to Tax plus TPA. However, these differences were not found to be statistically significant. This synergistic response was significantly enhanced by CsA (P < 0.01) (Fig. 4d; Tax + TPA). The response of the LTR to the combined treatment of Tax plus TPA was inhibited in the presence of ionomycin. However, in the presence of CsA the ionomycin-induced inhibition was recovered, as is shown in Fig. 4(d). This recovery resulted in much higher levels of lacZ expression than observed for Tax plus TPA treatment in the absence of ionomycin (P < 0.01).

Ionomycin inhibits virus expression by MT-2 cells

To investigate further the role of Ca\(^{2+}\) in the suppression of HTLV-1 expression, we examined the effects of ionomycin and the combined treatment of ionomycin transfection with pRSVtax; (d) TPA treatment following transfection with pRSVtax. Cells were prestimulated as indicated below each bar graph with medium alone (–), 5 μM-BAPTA/AM (B), 1 μg/ml CsA (C) or 2 mM-EGTA (E) prior to treatment with TPA or ionomycin. The results represent the means and standard deviations of three to five independent experiments. NT, no treatment.
plus CsA on extracellular virus production in MT-2 T cells. Table 1 shows the effect of Ca\(^{2+}\)-enhancing and -inhibiting compounds on virus production as measured by p24 detection in the supernatant. In the presence of 10 \(\mu M\) ionomycin a decrease in virus expression was clearly evident. Although toxic effects of ionomycin were sometimes observed at 5 and 10 \(\mu M\), the reduction in virus expression in these experiments did not correlate with the reduced numbers of viable cells. Increases in virus expression were not observed in cells treated with ionomycin plus CsA. However, in the presence of CsA, virus expression was not reduced by ionomycin at 10 \(\mu M\).

**Discussion**

The influence of T cell activation pathways on HTLV-1 expression has not been studied in depth. The activation of PKC by TPA has been shown to upregulate HTLV-1 expression positively. In this study we have investigated the influence of Ca\(^{2+}\)-dependent pathways induced by the calcium ionophore ionomycin on HTLV-1 expression. Using a Jurkat T cell line stably expressing a HTLV-1 LTR–lacZ construct we have demonstrated that ionomycin inhibits basal LTR responses and also inhibits trans-activation via TPA and/or Tax. The mechanism underlying this inhibition was investigated using several Ca\(^{2+}\)-blocking agents capable of inhibiting Ca\(^{2+}\)-mediated pathways at different levels. These included the agents EGTA and BAPTA/AM to chelate extracellular and intracellular Ca\(^{2+}\). The immunosuppressive drug CsA was employed to block a Ca\(^{2+}\)-sensitive T cell signal transduction pathway (Gunter et al., 1989; Kay et al., 1989; Lin et al., 1991; Mattila et al., 1990).

The chelation of extracellular or intracellular Ca\(^{2+}\) by the agents EGTA and BAPTA/AM, respectively, revealed that Ca\(^{2+}\) ions are indeed necessary for the response of the LTR to TPA-mediated activation. This result is not surprising as free Ca\(^{2+}\) is a co-requisite for translocation of PKC to the cell membrane (Blumberg, 1991). The increase in free Ca\(^{2+}\) within the cell induced by ionomycin did not overcome the effects of chelation on the TPA response. In contrast to TPA-mediated activation, trans-activation mediated by Tax did not demonstrate a dependence on Ca\(^{2+}\) mobilization as chelation of Ca\(^{2+}\) did not reduce Tax-induced lacZ expression. Therefore Tax does not appear to require Ca\(^{2+}\) as a co-factor in upregulating the activity of LTR-binding proteins. The inhibition of Tax-mediated trans-activation imposed by ionomycin was shown to be relieved by chelation of either intracellular or extracellular Ca\(^{2+}\). This suggests that the binding proteins elicited by Tax may be down-regulated or perhaps have a reduced affinity for the LTR in the presence of elevated levels of free Ca\(^{2+}\).

In our experiments, CsA upregulated HTLV-1 LTR-directed gene expression. Although ionomycin inhibited TPA- and Tax-mediated expression from the LTR, ionomycin and CsA used in concert enhanced LTR responses to TPA and Tax to above the levels obtained in the absence of ionomycin. These observations suggest that an increase in Ca\(^{2+}\) may have two effects. One effect results in the inhibition of HTLV-1 LTR trans-activation and this inhibition is in turn relaxed by CsA. The second effect appears to mediate an increase in trans-activation, but this is detected only when the negative effect is blocked by CsA. Several scenarios can be suggested to explain this result. Ionomycin may transmit a negative signal which could result in either a reduced affinity of transcription factors for the LTR or reduced expression of these molecules overall. This is not likely to be caused solely by elevated intracellular Ca\(^{2+}\) levels since the chelation of Ca\(^{2+}\) did not alleviate ionomycin-induced inhibition in all cases. In addition, the observation that CsA does not have a significant effect upon the mobilization of Ca\(^{2+}\) by ionomycin within the cell, as measured in Fluo-3/AM-labelled cells, suggests that the mode of action of the ionomycin-induced inhibition is likely to be the result of a CsA-suppressible T cell activation event(s). Protein-bound CsA, which is unable to enter the cell, can mediate an inhibition of T cell activation in a way similar to uncomplexed CsA (Cacalano et al., 1992). If CsA is able to act at the cell surface, then this activity may stimulate a different activation pathway to which the HTLV-1 LTR is responsive. Alternatively, CsA may prevent the activation of a Ca\(^{2+}\)-dependent pathway, and in this way block signals detrimental to HTLV-1 expression. The complementation of trans-activation by ionomycin plus CsA may result from suppression of an inhibitory pathway by CsA combined with a higher affinity of transcription factors for the LTR due to increased Ca\(^{2+}\) levels. Interaction of CsA with calcineurin, a Ca\(^{2+}\)-calmodulin-regulated phosphatase, has recently been shown to be the mechanism controlling CsA-induced immunosuppression. CsA blocks transcription of the IL-2 gene by inhibiting the phosphatase activity of calcineurin which is required for the binding activity of the transcription factors NF-AT and NF-IL2A (Clipstone & Crabtree, 1992).

Although ionomycin treatment reduced virus production from MT-2 cells, we were unable to demonstrate that the combination of ionomycin plus CsA had an upregulatory role on full virus expression. However, decreases in virus expression were not observed in cells treated with ionomycin plus CsA. As MT-2 cells are immortalized ATL cells and are unique in that they produce large amounts of HTLV-1, these cells may already be producing maximum levels of HTLV-1. In
addition, we have observed that treatment of MT-2 cells with TPA did not result in detectable increases in virus production as measured by antigen capture (data not shown).

Ionomycin has been shown to reduce the response of the IL-2 promoter to phorbol esters (Ullman et al., 1993) and this reduction was relieved by CsA. Phorbol ester activation of the IL-2 gene is mediated by the AP-1 binding element of the promoter. However, although the authors were able to demonstrate down-regulation at the level of gene expression, no differences in Jun binding to the AP-1 element were evident in the presence of ionomycin. Evidence for negative regulatory pathways and possibly specific negative response element(s) within the HTLV-LTR has been demonstrated by our findings and warrants further investigation. As ionomycin treatment affects basal LTR activity as well as the response of the LTR to trans-activation, the responsive elements concerned may be located in the U3 region of the LTR which houses Tax- and TPA-responsive elements, and possibly the U5 region, recently recognized to contain sequences controlling basal expression (Kashanchi et al., 1993).

In summary, our results show a Ca²⁺-related pathway is able to interfere with HTLV-1 expression and LTR-mediated gene expression. This inhibition affects transcriptional regulation mediated by both Tax and TPA and in both cases the inhibition is clearly relieved by CsA. In addition, we have shown that ionomycin and CsA can synergize in upregulation of the HTLV-1 LTR. An understanding of the pathways and transcriptional factors involved may provide new insight into the mechanisms which contribute to viral latency in the T cells of HTLV-1-infected individuals.

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References


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