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Activation of Protein Phosphatase-$2A_1$ by HIV-1 Vpr Cell Death Causing Peptide in Intact CD^{4+} T Cells and In Vitro

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Abstract HIV-1, the etiologic agent of human AIDS, causes cell death in host and non-host cells via HIV-1 Vpr, one of its auxiliary gene product. HIV-1 Vpr can also cause cell cycle arrest in several cell types. The cellular processes that link HIV-1 Vpr to the cell death machinery are not well characterized. Here, we show that the C terminal portion of HIV-1 Vpr which encompasses amino acid residues 71–96 (HIV-1 Vpr^{71-96}), also termed HIV-1 Vpr cell death causing peptide, is an activator of protein phosphatase-$2A_1$ when applied extracellularly to CD^{4+} T cells. HIV-1 Vpr^{71-96} is a direct activator of protein phosphatase-$2A_1$ that has been purified from CD^{4+} T cells. Full length HIV-1 Vpr by itself does not cause the activation of protein phosphatase-$2A_1$ in vitro. HIV-1 Vpr^{71-96} also causes the activation of protein phosphatase-$2A_0$ and protein phosphatase-$2A_1$ from brain, liver, and adipose tissues. These results indicate that HIV-1 can cause cell death of infected cells and non-infected host and non-host cells via HIV-1 Vpr derived C terminal peptide(s) which act(s) by cell penetration and targeting of a key controller of the cell death machinery, namely, protein phosphatase-$2A_1$. The activation of other members of the protein phosphatase-2A subfamily of enzymes which are involved in the control of several metabolic pathways in brain, liver, and adipose tissues by HIV-1 Vpr derived C terminal peptide(s) may underlie various metabolic disturbances that are associated with HIV-1 infection. J. Cell. Biochem. 94: 816–825, 2005.

Key words: HIV-1; Vpr; protein phosphatase-2A; cell death; metabolic disturbances

An important property of HIV-1, the etiologic agent of human AIDS, is its ability to cause cell death via one of its accessory gene product termed HIV-1 Vpr [Azad, 1997]. HIV-1 Vpr can also cause cell cycle arrest at the G_2 to M transition [Emerman, 1996]. The mechanism by which HIV-1 Vpr acts to cause cell death are not well understood. One possible mechanism by which HIV-1 Vpr may cause cell death of infected cells and non-infected host and non-host cells is through the release of free HIV-1 Vpr or one of its fragments in the extracellular environment. Soluble HIV-1 Vpr and related fragments have been detected in serum and cerebrospinal fluid of HIV-1 infected individuals [Levy et al., 1994]. Extracellular HIV-1 Vpr and one of its fragment have been shown to cause cell death in a number of different cell types. A peptide derived from the C terminus of HIV-1 Vpr has been shown to enter the cell and to cause death of human cells and yeast cells [Macreadie et al., 1996; Arunagiri et al., 1997; Piller et al., 1998; Huang et al., 2000; Patel et al., 2000; Jacotot et al., 2001].

Because protein phosphatase-$2A_1$ is involved as a negative controller of cell cycle progression and an activator/mediator of cell death, and okadaic acid which is a potent inhibitor of protein phosphatase-$2A_1$ prevents HIV-1 Vpr induced cell cycle arrest and cell death, it was suspected that protein phosphatase-$2A_1$ would

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be a target of HIV-1 Vpr [Meijer et al., 1986; Goris et al., 1989; He et al., 1995; Jowett et al., 1995; Re et al., 1995; Rogel et al., 1995; Shtrichman et al., 1999; Chiang et al., 2002] (H.Y. Lim Tung, unpublished results). Indeed, examination of the sequence of HIV-1 Vpr reveals that its C terminal portion resembles that of an activator of protein phosphatase-2A$_1$ [Tung et al., 1985a; Wong-Staal et al., 1987; Domenjoud et al., 1988]. Because other members of the protein phosphatase-2A subfamily of enzymes are involved in the control of insulin action, glycogen synthesis and breakdown, fatty acid synthesis, cholesterol synthesis, and lipid breakdown [Ingebritsen, 1983; Cohen, 1989], the targeting of protein phosphatase-2A subfamily of enzymes by HIV-1 Vpr can also explain the occurrence of various metabolic disturbances that are associated with HIV-1 infection. These include insulin resistance, hyperglycemia, hypertriglyceridemia, hypercholesterolemia, and also a special form of lipodystrophy which is characterized by wasting of peripheral fat (arms, legs, and face) but gain of central adiposity (abdomen, breast, and back) [Wood et al., 1993; Srinivasan and Begum, 1994; Gaussin et al., 1997; Carr et al., 1998; Shikuma et al., 1999; Dube, 2000; Graham, 2000; Qaqish et al., 2000].

Although the hypothesis that HIV-1 Vpr may target and activate protein phosphatase-2A$_1$ and other members of the protein phosphatase-2A subfamily of enzymes is a very attractive one, we were unable to demonstrate direct activation of protein phosphatase-2A$_1$ and other members of the protein phosphatase-2A subfamily of enzymes by full length HIV-1 Vpr. We could only show the activation of these enzymes by a HIV-1 Vpr:HIV-1 NCp7 complex [Tung et al., 1997] (H.Y. Lim Tung, unpublished results). However, a complex that consists of HIV-1 Vpr and HIV-1 NCp7 has not yet been identified in HIV-1 infected cells (H.Y. Lim Tung, unpublished results). In contrast to our published result, Hrimech et al. [2000] reported that full length HIV-1 Vpr can directly cause the activation of protein phosphatase-2A$_1$ in intact cells and in vitro by interacting with the B subunit of protein phosphatase-2A$_1$. However, because of the inability to reproduce the results of that article and also because of data fabrication, the article in question has had to be retracted in full by all the authors concerned [Hrimech et al., 2002]. In the present study, we have identified an alternative mechanism by which HIV-1 Vpr may interact with and modulate the activity of protein phosphatase-2A$_1$ and other forms of the protein phosphatase-2A subfamily of enzymes.

MATERIALS AND METHODS

Preparation of Proteins and Peptides

^{32}P-labeled phosphorylase a was prepared by phosphorylation of phosphorylase b with phosphorylase kinase as described by Cohen et al. [1988]. ^{32}P-labeled casein was prepared by phosphorylation of casein with protein kinase A as described by Tung [1986]. Protein phosphatase-1 was purified from rabbit skeletal muscle as described by Cohen et al. [1988]. Protein phosphatase-1 inhibitor-2 was purified from rabbit skeletal muscle as described by Yang et al. [1981]. Protein phosphatase-2A$_0$ and protein phosphatase-2A$_1$ from pig brain and liver were purified as described by Tung et al. [1985a]. Protein phosphatase-2A$_0$ and protein phosphatase-2A$_1$ from pig adipose tissue were highly purified by successive chromatography of pig subcutaneous adipose tissue extracts on DEAE Sepharose, poly-L-lysine agarose, Sephacryl S-300 HR, and thiophosphorylase-a-Sepharose-4B as described by Tung et al. [1985a]. Protein phosphatase-2B was purified from pig brain as described by Tung [1986]. Protein phosphatase-2C was highly purified from pig liver by successive chromatography of pig liver extracts on DEAE sepharose, casein agarose, and sephacryl S-300 HR. Full length HIV-1 Vpr and full length NCp7 were prepared from *Escherichia coli* strains that have been engineered to overexpress them and were kindly provided to us by Dr. L.-J. Zhao of St. Louis University School of Medicine [Zhao et al., 1994]. HIV-1 Vpr cell death causing peptide (referred as HIV-1 Vpr$^{71-96}$ in the text) and other peptides were prepared by chemical synthesis on an automated Solid Phase Peptide Synthesizer as recommended by the manufacturer and purified as described by Azzi et al. [1992]. The sequence of the peptides were confirmed by MALDI ToF MS. The sequence of HIV-1 Vpr$^{71-96}$ is HFRIGCRHSRIGVTRQRRARNGASRS. The sequence of full length HIV-1 Vpr is MEQAPEDQGPQREPYNDWTLELLEELKNEAVRHFPRIWLHSLGQHIYETYGDTWTGVEALIRILQQLLFIHFRIGCRHSRIGVTRQRRARNGASRS.

Assay of Protein Phosphatases

Protein phosphatase-1 was assayed as described by Cohen et al. [1988]. Protein phosphatase-2B was assayed as described by Tung [1986]. Protein phosphatase-2C was assayed as described by Tung [1986] except that Ca^{2+} and calmodulin were omitted. The assay of protein phosphatases-$2A_0$ and -$2A_1$ consisted of 0.02 ml of enzyme solution in 50 mM imidazole-Cl pH 7.2, 0.2 mM EGTA, 0.1% (v/v) 2-mercaptoethanol and 1 mg/ml bovine serum albumin (Assay Buffer), 0.01 ml of inhibitor-2 at 600 nM in Assay Buffer, 0.01 ml of protamine at 60 μg/ml in Assay Buffer or 0.01 ml of HIV-1 Vpr^{71-96} at different concentrations in Assay Buffer or 0.01 ml of Assay Buffer alone, 0.02 ml of ^{32}P-labeled phosphorylase a at 3 mg/ml in Assay Buffer containing 15 mM caffeine. The assay components were pre-incubated for 10 min prior to initiating the reaction with ^{32}P-labeled phosphorylase a. One unit of protein phosphatase activity is that amount of enzyme which catalyzes the release of 1 nmol of phosphate from ^{32}P-labeled substrate per min at 30°C.

Purification of Protein Phosphatase-$2A_1$ From CD^{4+} T Cells

Jurkat cells, a CD^{4+} T cell line, were grown in 2.4 L of RPMI 1640 medium containing 10% (v/v) fetal bovine serum and antibiotics. Cells were collected by centrifugation at 4,200 rpm in a low speed centrifuge for 10 min. The cells were homogenized in 80 ml of 50 mM imidazole-Cl pH 7.2, 2 mM EGTA, 2 mM EDTA, 0.1% (v/v) 2-mercaptoethanol, 0.2 mM PMSF, 1 mM benzamidine, 4 μg/ml aprotinin, 4 μg/ml leupeptin, 4 μg/ml pepstatin, 0.1 mM TLCK, 0.1 mM TPCK, 1 mM sodium orthovanadate, and 10% (v/v) glycerol by 40 strokes in a hand held glass homogenizer. The homogenate was centrifuged at 29,000 rpm in a high speed centrifuge for 30 min. The supernatant (i.e., the extract) was collected and loaded onto a DEAE Sepharose column (1.5 × 6 cm) equilibrated in 25 mM imidazole-Cl pH 7.2, 0.2 mM EGTA, 0.1% (v/v) 2-mercaptoethanol, 0.1 mM PMSF, 1 mM benzamidine, and 10% (v/v) glycerol (Buffer A). The column was washed with 50 ml of Buffer and then eluted with a 200 ml linear gradient of Buffer A to Buffer A plus 0.4M NaCl. The eluted fractions were then assayed for phosphorylase phosphatase activity in the presence of 100 nM inhibitor-2 and 10 μg/ml of protamine. Two major peaks of phosphorylase phosphatase were observed. The first and second peak represent protein phosphatase-$2A_0$ and protein phosphatase-$2A_1$, respectively. The second peak eluting at around 0.2M NaCl, representing the largest proportion of the total protein phosphatase activity and which became activated following treatment of CD^{4+} T cells with HIV-1 Vpr^{71-96} (Fig. 1), was collected and loaded onto a sephacryl S-300 HR column (2.5 × 90 cm) equilibrated in 50 mM imidazole-Cl pH 7.2, 0.2 mM EGTA, 0.1% (v/v) 2-mercaptoethanol, 0.2M NaCl, 0.1 mM PMSF, 1 mM benzamidine, and 10% (v/v) glycerol. The major activity eluting as a species of apparent molecular mass 300 kDa was collected, diluted 4-fold in Buffer A, and loaded onto a poly-L-lysine agarose column (1.5 × 4 cm) equilibrated in Buffer A. The column was washed with 50 ml of Buffer A and then eluted with a 200 ml linear gradient of Buffer A to Buffer A plus 0.5M NaCl. The active fractions eluting at ~0.30M NaCl salt concentration was pooled, concentrated by vacuum dialysis, and stored at −20°C in 50 mM imidazole-Cl pH 7.2, 0.2 mM EGTA in the presence of 50% (v/v) glycerol. The highly purified enzyme consisted of the characteristic subunits of protein phosphatase-$2A_1$, namely, the A, B, and C subunits as well as two other prominent protein bands which are under study (Fig. 1). The purification of the enzyme is

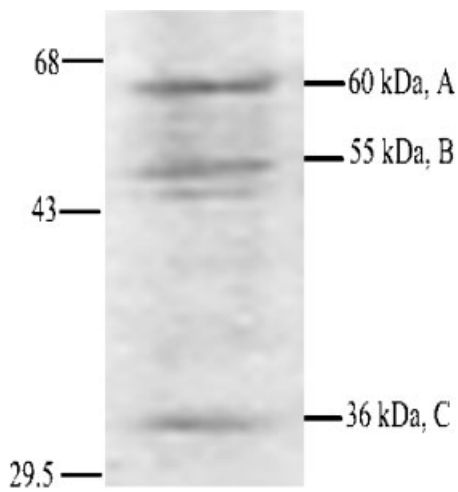

Fig. 1. SDS–PAGE of highly purified protein phosphatase-$2A_1$ from CD^{4+} T cells. Protein phosphatase-$2A_1$ purified from Jurkat cells as described in ''Materials and Methods'' was subjected to SDS–PAGE. The gel was stained with coomassie blue. Arrows indicate the positions and relative molecular masses of marker proteins (left) and of the three major proteins of protein phosphatase-$2A_1$ (right) in kDa.

TABLE I. Summary of the Purification of Protein Phosphatase-2A$_1$ From CD^{4+} T Cells

Steps	Activity (U)	Protein (mg)	Specific activity (U/mg)	Fold purification	Yield (%)
Extract	52.8	176	0.3	1	100
DEAE sepharose	21.1	1.98	10.7	35	40
Sephacryl S-300 HR	13.5	0.20	67.5	224	26
Poly-L-lysine agarose	3.0	0.011	272.7	908	6

summarized in Table I. The highly purified enzyme had a specific activity of 273 U per mg of protein. It is difficult to determine the activity of protein phosphatase-2A$_1$ in CD^{4+} T cell extract. However, assuming that it represented about 66% of total type 2A protein phosphatase activity in CD^{4+} T cell extract, the specific activity of the enzyme in extract was estimated to be 0.3 U/mg. The enzyme was therefore purified 908-fold. Like other previously characterized forms of protein phosphatase-2A, protein phosphatase-2A$_1$ from CD^{4+} T cells was not inhibited by inhibitor-2 but inhibited by okadaic acid and activated by protamine.

RESULTS

The C terminal portion of HIV-1 Vpr, HIV-1 Vpr$^{71-96}$, has previously been shown to penetrate CD^{4+} T cells and also to cause cell death of the latter [Macreadie et al., 1996; Arunagiri et al., 1997]. The sequence of HIV-1 Vpr$^{71-96}$ resembles that of an activator of protein phosphatase-2A subfamily of enzymes [Tung et al., 1985a; Wong-Staal et al., 1987; Domenjoud et al., 1988]. The effect of HIV-1 Vpr$^{71-96}$ on the activity of protein phosphatase-2A$_1$ in intact cells was therefore investigated. Jurkat cells, a CD^{4+} T cell line, were grown in RPMI 1640 in 10% (v/v) fetal bovine serum and then treated or not with 1,000 nM HIV-1 Vpr$^{71-96}$ which was added directly to the culture medium in the presence of 0.001% (v/v) DMSO. Extracts were then prepared as described in "Materials and Methods" and chromatographed on DEAE Sepharose column, an efficient procedure for the separation of protein phosphatase-2A$_1$. Protein phosphatases were then assayed in the presence of protein phosphatase-1 inhibitor-2, a very effective method for the determination of the different forms of protein phosphatase-2A [Tung et al., 1985a]. As shown in Figure 2, protein phosphatase-2A$_1$ which elutes at around 0.18–0.2M NaCl salt concentration became activated when cells were treated with HIV-1 Vpr$^{71-96}$. Treatment of Jurkat cells with HIV-1 Vpr$^{71-96}$ was accompanied by cell death of the former. After 4 h of incubation with HIV-1 Vpr$^{71-96}$, almost 60% of the treated cells became non-viable as determined by tryphan blue staining (Fig. 3A). Examination of CD^{4+} T cells treated with HIV-1 Vpr$^{71-96}$ by phase contrast microscopy showed that they died as a result of oncolysis (Fig. 3B).

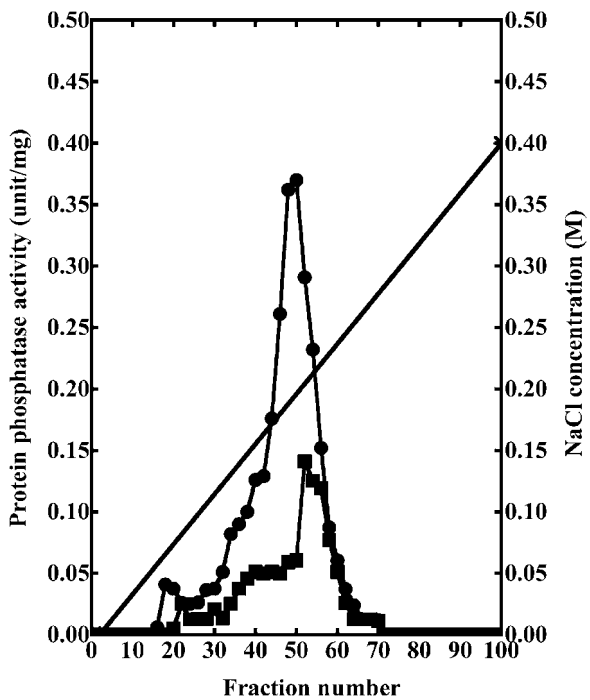

Fig. 2. Effect of HIV-1 Vpr$^{71-96}$ on the activity of protein phosphatase-2A$_1$ in intact CD^{4+} T cells. Jurkat cells (a CD^{4+} T cell line) were grown in RPMI 1640 and 10% (v/v) fetal bovine serum. Sixteen hundred milliliters of cell suspension (10^6 cells per ml) were split into two flasks and one was treated with HIV-1 Vpr$^{71-96}$ for 1 h (circles) while the other was not (squares). The cells from each flask were collected by centrifugation, washed with 50 mM imidazole-Cl pH 7.2 plus 150 mM NaCl, and then homogenized in homogenization buffer as described in "Materials and Methods." The homogenate from each flask was chromatographed on a DEAE Sepharose column and the eluted fractions were assayed for type 2A protein phosphatase activity as described in "Materials and Methods." Similar results were obtained in three independent experiments.

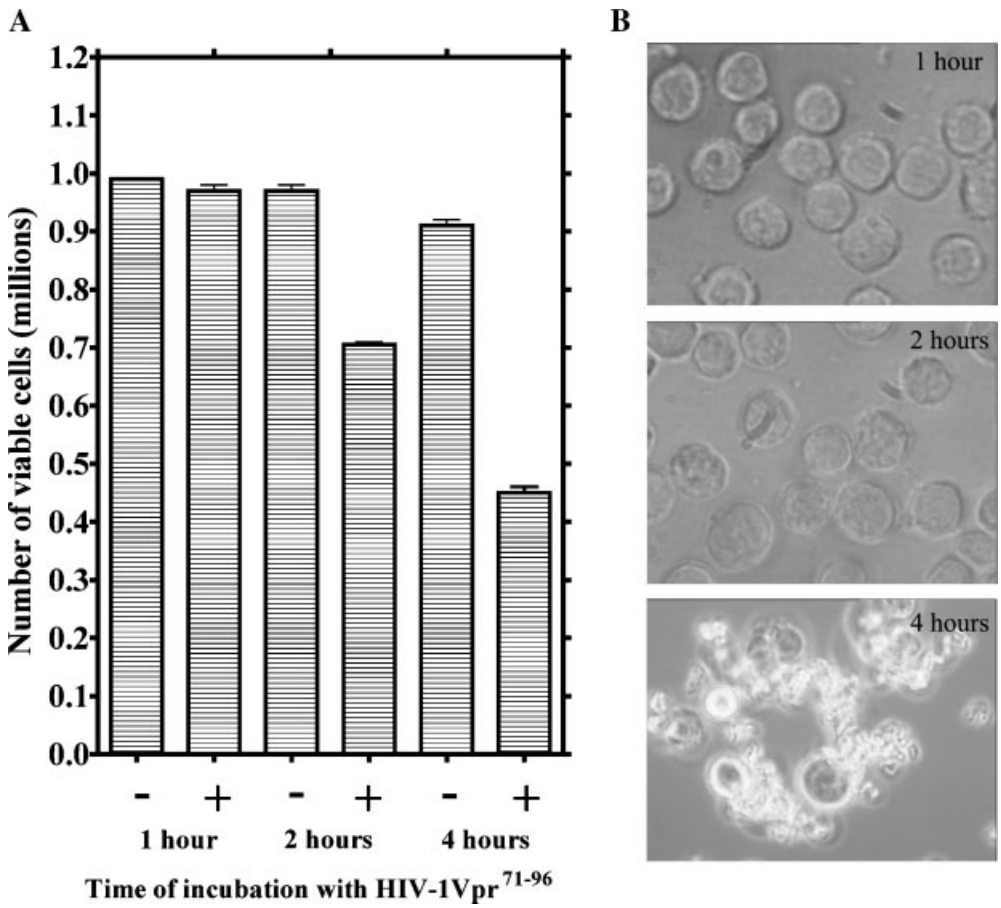

Fig. 3. Induction of cell death by HIV-1 Vpr^{71-96}. **Panel A**: Jurkat cells (a CD^{4+} T cell line) were grown in RPMI 1640 and 10% (v/v) fetal bovine serum. Cells (10^6 cells per ml) were treated with 1,000 nM HIV-1 Vpr cell death causing peptide or HIV-1 Vpr^{71-96}. Cell viability was then determined by counting the cells following tryphan blue staining after 1, 2, and 4 h of incubation. **Panel B**: Following treatment with HIV-1 Vpr^{71-96} for 1, 2, and 4 h as indicated, Jurkat cells (10^6/ml) were washed with RPMI plus 2% fetal bovine serum and then deposited onto glass slides by cytospinning. Phase contrast images were then obtained with white light emission on a Zeiss Axioplan 2 imaging universal microscope (Thornwood, NY). The images were captured and stored digitally with the aid of Northern Eclipse Version 6 software (Zeiss). Similar results were obtained in three independent experiments.

The effect of HIV-1 Vpr^{71-96} on the activity of protein phosphatase-$2A_1$, the major form of protein phosphatase-2A in CD^{4+} T cells, was determined in vitro. HIV-1 Vpr^{71-96} activated protein phosphatase-$2A_1$ by sevenfold with half maximal activation occurring at around 300 nM. The effect of HIV-1 Vpr^{71-96} was biphasic. At a concentration of above 1,000 nM, there was inhibition of protein phosphatase-$2A_1$ by HIV-1 Vpr^{71-96}. This phenomenon is observed with other activators of type 2A protein phosphatases (Fig. 4) [Tung et al., 1985a,b]. A peptide, which encompasses amino acid residues 1–50 of HIV-1 Vpr had negligible effect on the activity of protein phosphatase-$2A_1$ (data not shown). Recombinant full length HIV-1 Vpr that was overexpressed in bacteria had no significant effect on the activity of protein phosphatase-$2A_1$ (Fig. 4). These results suggest that within the full length HIV-1 Vpr, the activating domain which is localized in the carboxyl terminal portion is masked by an interfering or inhibitory domain. Figure 5 shows that purified protein phosphatase-$2A_1$ from CD^{4+} T cells can interact directly with HIV-1 Vpr^{71-96} bound to Sepharose.

The effect HIV-1 Vpr^{71-96} on the activities of other major forms of protein phosphatase, namely, protein phosphatase-1, protein phosphatase-2B, and protein phosphatase-2C were determined. Figure 6 shows that the effect of HIV-1 Vpr^{71-96} was quite specific. It had negligible effect on the activities of protein phosphatases-1, -2B, and -2C. We have previously

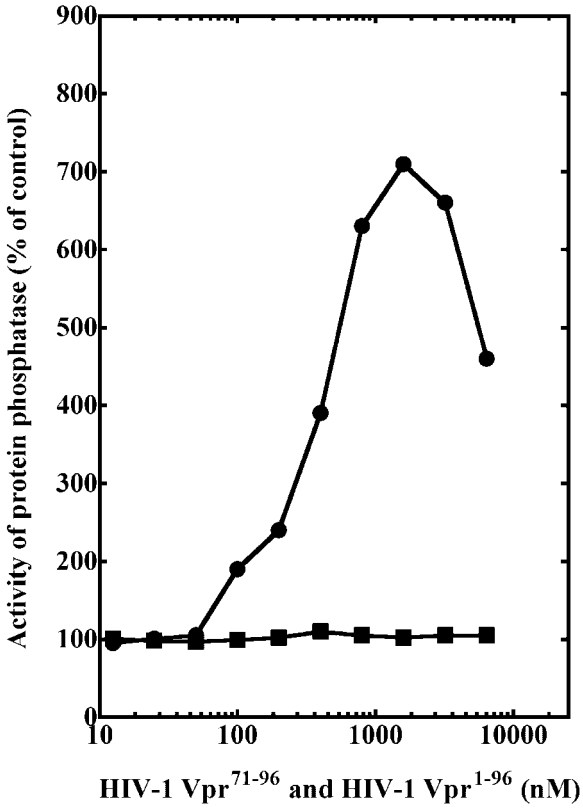

Fig. 4. HIV-1 Vpr$^{71-96}$ and full length HIV-1 Vpr on activity of purified protein phosphatase-2A$_1$ from CD^{4+} T cells. Protein phosphatase-2A$_1$ purified from CD^{4+} T cells as described in "Materials and Methods" was assayed in the presence of various concentrations of HIV-1 Vpr$^{71-96}$ (circles) or full length HIV-1 Vpr (squares). Hundred percent protein phosphatase-2A$_1$ activity is equivalent to 0.005 U. Similar results were obtained in three independent experiments.

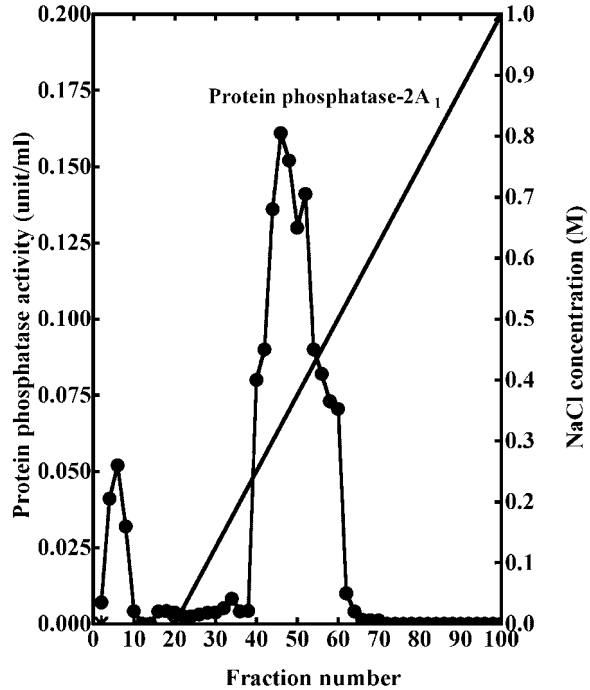

Fig. 5. Interaction between protein phosphatase-2A$_1$ from CD^{4+} T cells and HIV-1 Vpr$^{71-96}$-Sepharose. Protein phosphatase-2A$_1$ (3 U) purified from Jurkat cells as described in "Materials and Methods" was chromatographed on a HIV-1 Vpr$^{71-96}$-Sepharose column (1.0 × 1.5 cm) equilibrated in 50 mM imidazole-Cl pH 7.2, 0.2 mM EGTA, 0.1% (v/v) 2-mercaptoethanol, and 10% (v/v) glycerol. The column was eluted as indicated with buffer plus NaCl and the fractions (1 ml) were assayed for protein phosphatase-2A$_1$ activity as described in "Materials and Methods." Similar results were obtained in two independent experiments.

reported that another HIV-1 encoded protein termed HIV-1 NCp7 also causes the activation of protein phosphatase-2A$_0$ from pig brain [Tung et al., 1997]. Figure 7 shows that HIV-1 NCp7 could also cause the activation of protein phosphatase-2A$_1$ from CD^{4+} T cells. However, HIV-1 NCp7 was a less efficient activator of protein phosphatase-2A$_1$ from CD^{4+} T cells than HIV-1 Vpr$^{71-96}$, with 3.7-fold maximum activation and half maximal activation occurring at 2,500 nM. Unlike HIV-1 Vpr$^{71-96}$, HIV-1 NCp7 cannot act extracellularly to cause the activation of protein phosphatase-2A$_1$ in CD^{4+} T cells (data not shown).

Several members of the protein phosphatase-2A subfamily of enzymes were originally identified as enzymes, which are involved in the regulation of glycogen synthesis and breakdown, fatty acid synthesis, cholesterol synthesis, and triacylglycerol breakdown. Members of the protein phosphatase-2A subfamily are also involved in the control of several key signal transduction pathways in the brain [Nishi et al., 2002]. Deregulation of protein phosphatase-2A subfamily of enzymes may, therefore, contribute to various metabolic disturbances that are observed in HIV-1 infected individuals. The effect of HIV-1 Vpr$^{71-96}$ on the activities of protein phosphatase-2A$_0$ and protein phosphatase-2A$_1$ from pig brain, pig liver, and pig adipose tissues were determined. Figure 8 shows that protein phosphatase-2A$_0$ and protein phosphatase-2A$_1$ from these tissues were also activated by HIV-1 Vpr$^{71-96}$ in vitro.

DISCUSSION

The present study has identified a mechanism by which HIV-1 acting via its accessory gene product HIV-1 Vpr may cause cell death. A

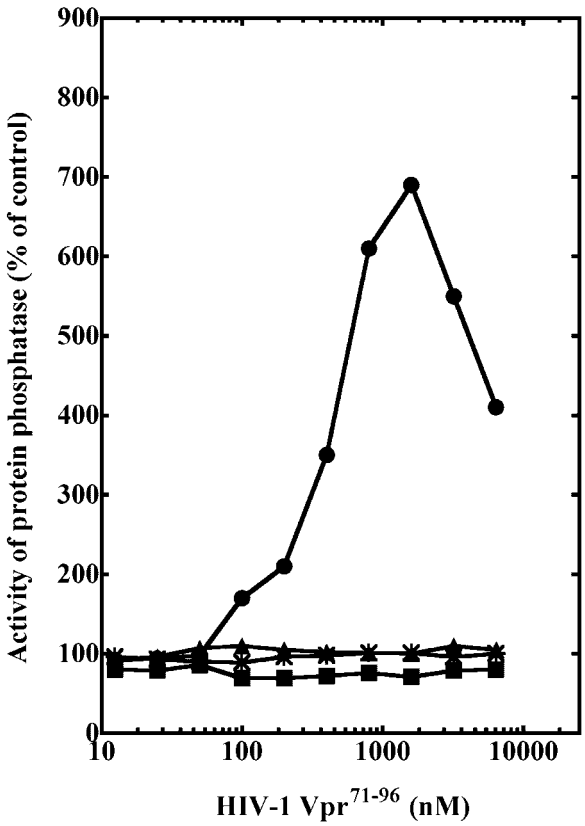

Fig. 6. Effect of HIV-1 Vpr^{71-96} on the activities of protein phosphatase-1, protein phosphatase-$2A_1$, protein phosphatase-2B, and protein phosphatase-2C. Protein phosphatase-1 from rabbit skeletal muscle (squares), protein phosphatase-$2A_1$ from CD^{4+} T cells (circles), protein phosphatase-2B from pig brain (triangles), and protein phosphatase-2C from pig liver (stars) were assayed in the presence of various concentrations of HIV-1 Vpr^{71-96}. Hundred percent protein phosphatase activity is equivalent to 0.005 U. Similar results were obtained in three independent experiments.

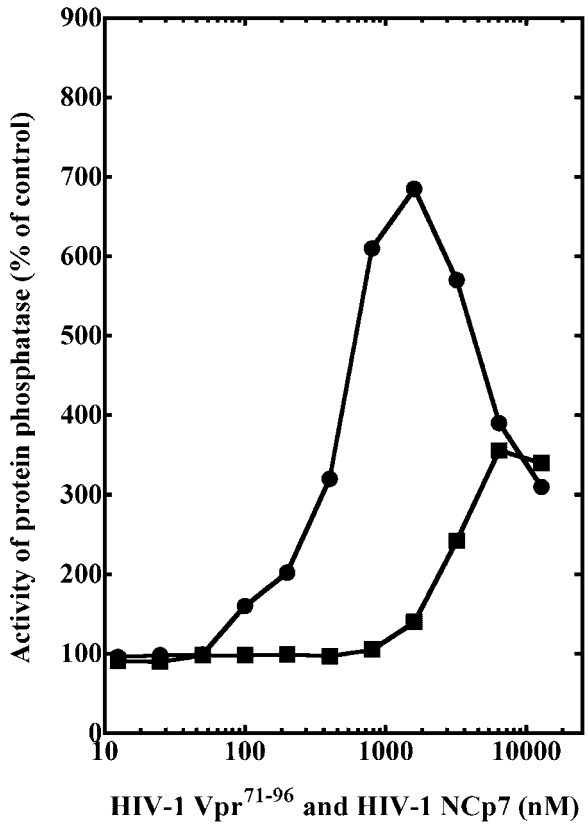

Fig. 7. Effect of HIV-1 Vpr^{71-96} and HIV-1 NCp7 on the activity of protein phosphatase-$2A_1$ from CD^{4+} T cells. Protein phosphatase-$2A_1$ purified from CD^{4+} T cells was assayed in the presence of various concentration of HIV-1 Vpr^{71-96} (circles) and HIV-1 NCp7 (squares). Hundred percent protein phosphatase activity is equivalent to 0.005 U. Similar results were obtained in three independent experiments.

peptide derived from the C terminal portion of HIV-1 Vpr, that is able to cause cell death in CD^{4+} T cells and also of directly activating protein phosphatase-$2A_1$ in CD^{4+} T cells, has been identified. Protein phosphatase-$2A_1$ can act as a negative controller of cell cycle progression and a positive regulator/mediator of cell death [Meijer et al., 1986; Goris et al., 1989; Shtrichman et al., 1999; Chiang et al., 2002]. The results of this article show that full length HIV-1 Vpr does not cause the activation of protein phosphatase-$2A_1$. We have shown that purified protein phosphatase-$2A_1$ holoenzyme can interact directly with an affinity column that consists of HIV-1 Vpr^{76-96} covalently bound to sepharose. HIV-1 Vpr^{71-96} can also cause the activation of protein phosphatase-$2A_0$ and protein phosphatase-$2A_1$ from brain, liver, and adipose tissues. These results indicate that HIV-1 Vpr^{71-96} does not interact with the B subunit of protein phosphatase-$2A_1$ since protein phosphatase-$2A_0$ does not contain a B subunit but a B′ subunit which is quite distinct from the former [Tung et al., 1985a]. It is most likely that HIV-1 Vpr^{71-96} interacts with the A or the C subunits or both since protein phosphatase-$2A_0$ and protein phosphatase-$2A_1$ share both of the these subunits.

Works on HIV-1 Vpr have concentrated on identifying its role in the virion and the host cells. Azad [1997] has suggested that extracellular HIV-1 Vpr may have an important role in AIDS progression. These workers showed that a peptide that encompasses residues 71–96 of HIV-1 Vpr could act extracellularly and was sufficient for causing cell death [Macreadie

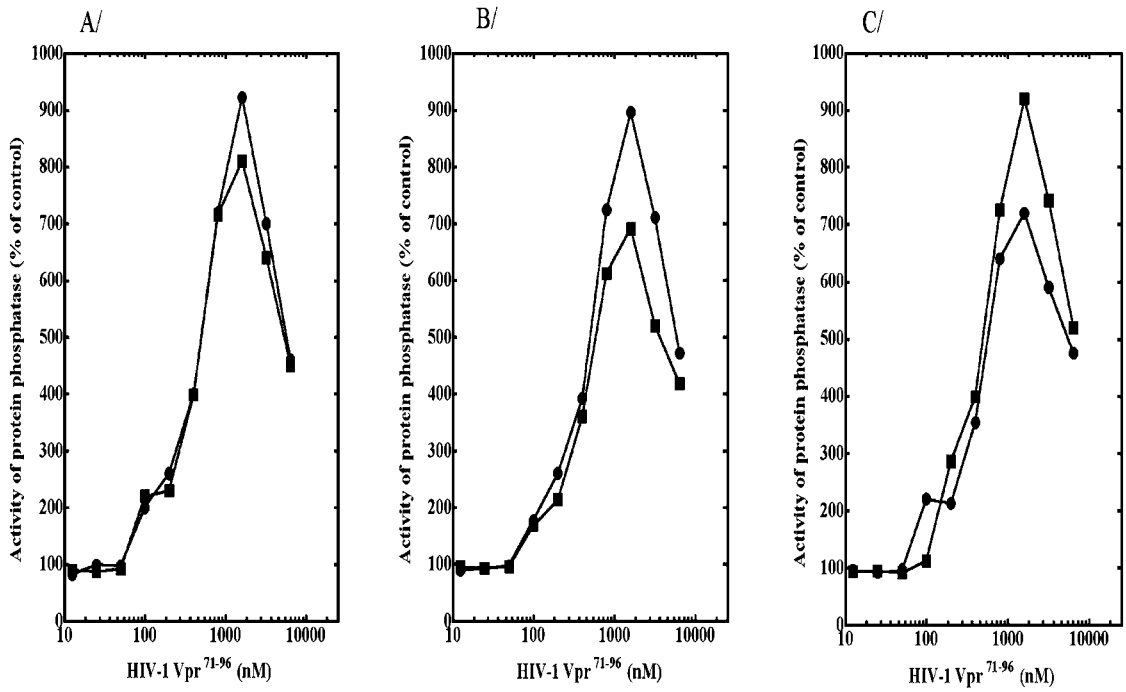

Fig. 8. Effect of HIV-1 Vpr$^{71-96}$ on the activities of protein phosphatase-2A$_0$ and protein phosphatase-2A$_1$ from various tissues. Protein phosphatase-2A$_0$ (squares) and protein phosphatase-2A$_1$ (circles) from pig brain (**panel A**), pig liver (**panel B**), and pig adipose tissue (**panel C**) were assayed in the presence of various concentration of HIV-1 Vpr$^{71-96}$. Hundred percent protein phosphatase activity is equivalent to 0.005 U. Similar results were obtained in three independent experiments.

et al., 1996; Arunagiri et al., 1997]. The present study shows that only HIV-1 Vpr$^{71-96}$, and not the full length HIV-1 Vpr, is capable of activating protein phosphatase-2A$_1$, an important controller of cell cycle progression and an activator/mediator of the cell death machinery of the cell. It is possible that full length HIV-1 Vpr is cleaved in HIV-1 infected cells in order to generate a HIV-1 Vpr derived peptide which is released in the blood stream where it can act on infected cells and also on non-infected host and non-host cells. Soluble HIV-1 Vpr and HIV-1 Vpr fragments have been identified in serum and cerebrospinal fluid of HIV-1 infected individuals [Levy et al., 1994]. Our recent study shows that HIV-1 Vpr is cleaved to a number of fragments in HIV-1 infected CD^{4+} T cells (manuscript in preparation). The signal transduction pathway that links activation of protein phosphatase-2A$_1$ to cell death in CD^{4+} T cells remains to be delineated.

HIV-1 infection is often accompanied by various metabolic disturbances which include insulin resistance, hyperglycemia, hypertriglyceridemia, hypercholesterolemia, and also the syndrome of lipodystrophy [Carr et al., 1998; Shikuma et al., 1999; Dube, 2000; Graham, 2000; Qaqish et al., 2000]. The finding that HIV-1 Vpr$^{71-96}$ can act extracellularly, traverse the cell membrane, and target several members of the protein phosphatase-2A subfamily of enzymes, is of interest because several forms of protein phoaphatase-2A were initially characterized as enzymes which regulate several metabolic pathways including insulin action, glycogen synthesis and breakdown, fatty acid synthesis, cholesterol synthesis, and lipolysis. We hypothesize that HIV-1 Vpr derived C terminal peptide(s) which are released in the blood stream from HIV-1 infected cells can also act on liver and adipose tissues. Activation of the forms of protein phosphatase-2A that are involved in the control of the above metabolic pathways by HIV-1 Vpr derived C terminal peptide(s) in liver and adipose tissues will result in the deregulation of the rate limiting enzymes concerned, thereby contributing to the metabolic disturbances that are associated with HIV-1 infection. An uncharacterized form of protein phosphatase-2A is known to be involved in

the dephosphorylation of DARPP-32, a key controller of brain function [Nishi et al., 2002]. Activation of the relevant form(s) of protein phosphatase-2A in brain tissue by HIV-1 Vpr derived C terminal peptide(s) can potentially contribute to various brain abnormalities in HIV-1 infected individuals.

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