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The glucocorticoid receptor type II complex is a target of the HIV-1 *vpr* gene product

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ABSTRACT **The *vpr* gene of human immunodeficiency virus type 1 (HIV-1) encodes a 15-kDa virion-associated protein that functions as a regulator of cellular processes linked to the HIV life cycle. We report the interaction of a 41-kDa cytosolic viral protein R interacting protein 1 (Rip-1) with Vpr *in vitro*. Rip-1 displays a wide tissue distribution, including relevant targets of HIV infection. Vpr protein induced nuclear translocation of Rip-1, as did glucocorticoid receptor (GR)-II-stimulating steroids. Importantly, Vpr and Rip-1 coimmunoprecipitated with the human GR as part of an activated receptor complex. Vpr complementation of a *vpr* mutant virus was also mimicked by GR-II-stimulating steroids. Vpr and GR-II actions were inhibited by mifepristone, a GR-II pathway inhibitor. Together these data directly link the activity of the *vpr* gene product to the glucocorticoid steroid pathway and provide a biochemical mechanism for the cellular and viral activity of Vpr, as well as suggest that a unique class of antivirals, which includes mifepristone (RU486), may influence HIV-1 replication.**

Human immunodeficiency virus (HIV)-1, HIV-2, and simian immunodeficiency virus contain, in addition to the canonical *gag/pol/env* genes, additional small open reading frames encoding gene products, including the 96-amino acid 15-kDa virion-associated HIV-1 Vpr gene product. The conservation of the *vpr* open reading frame in primate lentiviral evolution suggests that *vpr* is critical to viral function. The incorporation of *vpr* into virions (1, 2), as well as its cellular colocalization with *gag* (3–5), has led to speculation that *vpr* performs a structural role in the virus particle. However, *vpr*-deletion mutant viruses produce virions that appear normal in electron micrographs (6), and *vpr*-deletion mutant viruses remain infectious with somewhat lower replication kinetics in many $CD4^+$ T-cell lines (7–10). It has been suggested that delivery of Vpr into cells by virus could increase cellular permissiveness to early events in virus replication (11).

Expression of the *vpr* gene of HIV-1 is sufficient to induce growth arrest and cellular differentiation (11), and HIV-1 replication in macrophages can be inhibited by *vpr* antisense deoxyribonucleotides (12). *vpr*-deletion mutants are poorly infectious in myeloid lines *in vitro* (13, 14), indicating an important function for *vpr* in infection of this lineage. In trans extracellular Vpr protein increases virus replication in T lymphocytes and monocyte/macrophages *in vitro* (15), although the effects on monocytes/macrophages were clearly greater. nef^-, vpr^- mutant simian immunodeficiency virus replicated poorly in nonhuman primates (16). Purified Vpr protein isolated from HIV^+ patient serum reactivates HIV-1 replication in nonproductively infected cells (17).

Transfection studies have shown that Vpr is a weak transactivator of the HIV long terminal repeat and several other heterologous viral promoters, including human T-lymphotropic virus 1, Epstein–Barr virus, and cytomegalovirus (10). These observations and the fact that *vpr* is packaged into virions suggest that *vpr* may function as an activator of viral mRNA transcription in the pretranscriptional *tat*-independent stage (18).

We used a biologically active recombinant Vpr protein as a ligand to identify its cellular targets. Here we report that modulation of virus infection and cell status by Vpr is through direct interaction with a cellular protein, which can associate with the glucocorticoid receptor (GR) transcriptional complex. Anti-glucocorticoid agents could abrogate the above-described Vpr functions, suggesting that this pathway could be relevant for limiting HIV infection.

MATERIALS AND METHODS

Cell Culture and Virus Preparation. The U937 human promyelocytic cell line and the human embryonal rhabdomyosarcoma (RD) cell line were from the American Type Culture Collection and maintained as described (11, 17). Viruses were prepared as described (15, 17).

Ligand Blot. About 3×10^6 cells were washed in phosphate-buffered saline (PBS) and lysed in lysis buffer [150 mM NaCl/50 mM Tris, pH 8.0/0.5% (vol/vol) Triton X-100/protease inhibitors], as described (19). Cell lysates were incubated on ice for 10 min with frequent mixing, then centrifuged at $12,000 \times g$ for 6 min. The soluble (a crude preparation consisting of the suspension of both cytoplasmic, as well as cytoplasmic membrane and plasma membrane, fractions) and the insoluble fractions (organelle plus nuclear fraction) were run on SDS/12% PAGE and transferred to poly(vinylidene difluoride) membranes (Millipore) (20). Membranes were blocked by incubation with 5% nonfat dry milk dissolved in Tris buffer saline (TBS)/0.05% Tween 20 and washed extensively. These membranes were incubated with either purified Vpr protein (≈50 ng/ml) or an irrelevant protein (bovine serum albumin, 50 ng/ml). Membranes were washed and detected with anti-Vpr antiserum 808, followed by ^{125}I-labeled protein G (NEN). The membranes were blotted, air-dried, and exposed to film for 12 hr at −80°C.

Rip-1 Isolation. A Vpr–CNBr column was constructed by coupling purified recombinant Vpr to beads (Sigma) as per the manufacturer's specifications. This column was loaded with the U937 cell lysate for 1 hr at 4°C and washed extensively in PBS. Elution was by equilibration with 10 mM sodium phosphate buffer, pH 6.8, followed by 100 mM glycine, pH 2.5. The

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Abbreviations: HIV, human immunodeficiency virus; GR, glucocorticoid receptor; RD cells, rhabdomyosarcoma cells; hsp, heat shock protein; PMA, phorbol 12-myristate 13-acetate; Rip-1, R interacting protein 1; DSS, disuccinimidyl suberate; DTSSP, 3,3′-dithiobis(sulfosuccinimidylpropionate).

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eluted fractions were neutralized with 1/20th vol of 1 M sodium phosphate, pH 8.0, and stored at 4°C in protease inhibitor-containing buffer.

Antibodies. The rabbit anti-Vpr peptide serum (808, Bryan Cullen, Duke University), human anti-Gag p24 (V7.8, Evan Hersch, University of Arizona), and sheep anti-p24 antibody (Michael Phelan, Food and Drug Administration) were obtained through the National Institutes of Health AIDS Research and Reference Reagent Program. Antibodies were used as described (15, 17, 21).

Crosslinking. Fractions containing proteins were resuspended in PBS. This material was exposed to either the noncleavable crosslinking agent disuccinimidyl suberate (DSS) or the reversible crosslinking agent 3,3′-dithiobis(sulfosuccinimidylpropionate) (DTSSP; Pierce), as described (19).

Expression and Purification of Recombinant HIV-1 Vpr. Expression of Vpr in insect cells has been described (15, 17). For purification, Triton X-100 at 0.05% (vol/vol; final concentration) was added to the baculovirus supernatants that were passed over a rabbit anti-Vpr column (17). After being washed, the columns were eluted as follows. Three bed volumes of the preelution buffer 10 mM phosphate buffer/0.05% Triton X-100, pH 8.0, were passed through the column, followed by the elution buffer 10 mM triethanolamine/0.05% Triton X-100, pH 11.5. The eluate was collected and neutralized with 1/20th vol of 1 M sodium phosphate buffer, pH 6.8/0.05% Triton X-100.

Immunoprecipitation of GR Complexes. U937 cells were stimulated with the indicated agents and exposed to crosslinking agents (DSS or DTSSP) and subsequently lysed. The lysates were centrifuged at 12,000 × *g* for 10 min, and immediately used for immunoprecipitation (19) using a specific mouse anti-human GR reagent (Affinity BioReagents, Jersey City, NJ) covalently coupled to protein G-agarose beads (GIBCO/BRL) using dimethyl pimelimidate·2HCl (Pierce) (20). Alternatively, U937 cells were lysed and then stimulated with the indicated agents. The stimulated lysates were then exposed to crosslinking reagents, and these preparations were used in immunoprecipitation analysis.

RESULTS

Identification of Vpr Cellular Binding Proteins in Cell Lysates. We investigated the interaction of Vpr with cellular proteins that might couple Vpr to intracellular signaling pathways. R interacting protein 1 (Rip-1) was detected by using either recombinant (data not shown) or virally derived Vpr protein (Fig. 1*A*). We detected a 41-kDa protein (Rip-1) from the soluble fractions but not in the Triton X-100-insoluble fraction. Rip-1 and Vpr coeluted from a Vpr-specific immunoaffinity column (Fig. 1*B*). Next, a Vpr–CNBr-activated Sepharose column loaded with the Triton X-100-soluble fraction of RD cell lysates was eluted to yield a single major protein (>95% purity). This protein corresponded in size to Rip-1 and reacted with Vpr in a ligand blot system similarly to the protein found in total cell lysate (Fig. 1*C*). Furthermore, Rip-1 and Vpr could be reversibly crosslinked to a 58-kDa heterodimeric complex that reacted with the Vpr-specific antibody in immunoblot or ligand blots (Fig. 1*D*). Rip-1 represents a cellular target for Vpr.

Cellular Trafficking Studies of Rip-1 in Response to Vpr and Other Stimuli. Cellular components were segregated using a Triton X-100 lysis procedure and subjected to ligand blotting, as described in *Materials and Methods*, in the presence or absence of various agents. Rip-1 was found consistently present in the crude soluble fraction before Vpr exposure (Fig. 2). Upon exposure of cells to Vpr protein or HIV-1 virus, but not to phorbol 12-myristate 13-acetate (PMA), which activates HIV expression, at least in part, through protein kinase C activation, Rip-1 was seen to translocate from the crude cytoplasmic to the nuclear insoluble fraction. In addition, an infectious *vpr*-deletion mutant HIV-1 virus [HIV-1 NL43Δvpr (11)] could not induce Rip-1 translocation after infection of U937 cells. Importantly, Vpr was observed to cotranslocate to the crude nuclear fraction with Rip-1. Coadministration of HIV-1 NL43Δvpr and exogenous Vpr protein induced the nuclear translocation of Rip-1 by 12 hr after initial exposure of the cells.

The viral replication kinetics also correlated with cotranslocation of Rip-1 and Vpr. U937 cells infected with NL43 produced detectable levels of virus typically at day 4 after infection. Rip-1 cotranslocated to the nuclear fraction with Vpr on day 3 after infection. U937 cells infected with NL43Δvpr failed to establish a productive infection. When the nonproductively infected U937 cells were exposed to Vpr protein, Rip-1 translocated 12 hr later, and virus was first detected in the medium 36 hr after the initial exposure to Vpr. These data support functional coupling of Vpr and Rip-1 in HIV infection.

Nuclear Translocation of Rip-1 Is Induced by Hydrocortisone and Dexamethasone in the Absence of Vpr. Possibly Rip-1 is a carrier protein that translocates Vpr to the cell nucleus.

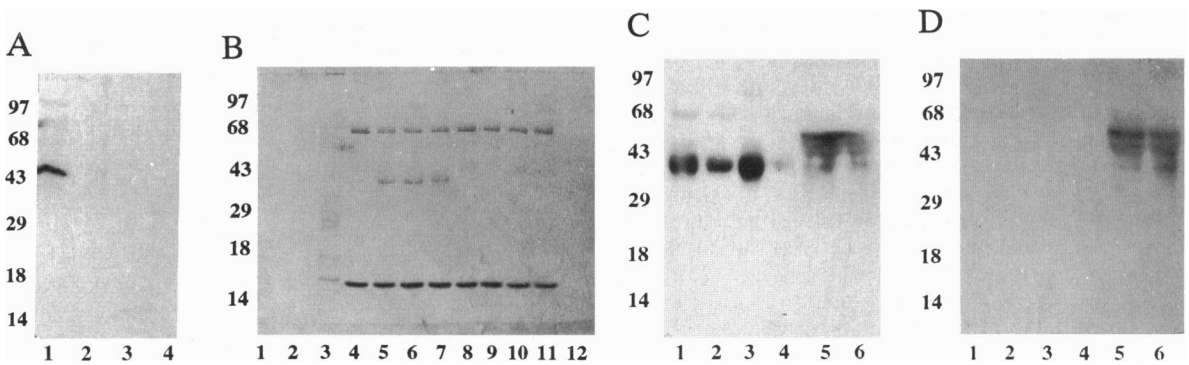

FIG. 1. Identification of a 41-kDa protein (Rip-1) by Vpr ligand-immunoblot. (*A*) Thirty microliters of Triton X-100 crude soluble fractions (odd-numbered lanes) or Triton X-100 crude insoluble fractions (even-numbered lanes) derived from RD cells was immunoblotted and probed with virally derived Vpr protein lanes (lanes 1 and 2) or irrelevant protein (lanes 3 and 4). (*B*) Coelution of Vpr and Rip-1 from an immunoaffinity column. A U937 (10^7 cells) soluble crude fraction was run over an anti-Vpr immunoaffinity column previously loaded with recombinant Vpr. After elution the gel was loaded with 10 μl of 1-ml sequential fractions of the column elution, and proteins were detected by silver-stain reactivity. An apparently nonspecific 68-kDa band is frequently observed after elution of control, as well as experimental, fractions from this column. (*C* and *D*) Crosslinking of Rip-1/Vpr complexes. Lanes: 1 and 2, 10 μl and 1 μl of Rip-1 isolated through use of a Vpr–CNBr-Sepharose column that yields a single predominant Rip-1 band; 3 and 4, 10 μl and 1 μl of total cell lysate (Triton X-100-soluble fraction of U937 cell lysate); 5 and 6, Rip-1 + Vpr protein DSS-crosslinked heterodimers. In *C* the protein was detected with Vpr and anti-Vpr antibody, and in *D* it was detected with the anti-Vpr antibody.

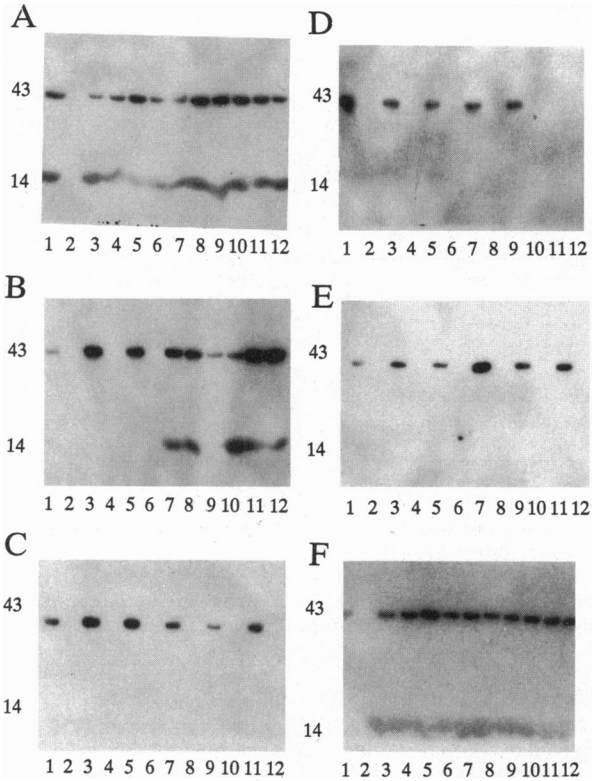

FIG. 2. Induction of Rip-1 translocation by various agents. U937 cells were exposed to recombinant Vpr protein (*A*), HIV-1 NL43 (wild-type) virus (*B*), HIV-1 NL43Δvpr virus (*C*), control supernatants, lacking Vpr protein (*D*), PMA (*E*), and HIV-1 NL43Δvpr with exogenous Vpr protein (*F*). Samples were Triton X-100 cellular lysates, soluble fractions (odd-numbered), or insoluble fractions (even-numbered). Samples were collected at successive time points; 12 hr (lanes 1 and 2), at 24 hr (lanes 3 and 4), at 48 hr (lanes 5 and 6), at 72 hr (lanes 7 and 8), at 96 hr (lanes 9 and 10), and at 120 hr (lanes 11 and 12). Nuclear translocation of Rip-1 was seen in *A*, *B*, and *F* [in the presence of Vpr protein, HIV-1 NL43 (wild type), and HIV-1 NL43Δvpr plus Vpr protein, respectively]. Vpr and Rip-1 were identified by using Vpr protein as the probe for Rip-1 and anti-Vpr as the specific detection agent.

There, Vpr could exert its biological function. Alternatively, Vpr could function as the ligand for a protein involved in a distinct cellular signaling pathway; the cellular trafficking properties we observed for Rip-1 share characteristics with members of the glucocorticosteroid receptor superfamily (for review, see refs. 22–25) and translocation/transcription complex. Type II GRs, and their accessory proteins, translocate from the cytoplasm to the nucleus upon binding to their ligand. We tested the effects of steroid hormones on Rip-1 translocation. Although minor loading differences were seen in a particular assay, the gels were run a minimum of three times with similar results. Vpr induced Rip-1 translocation (Fig. 3 *A* and *B*, lanes 1 and 2). Neither control supernatants (Fig. 3 *A* and *B*, lanes 3 and 4), PBS (Fig. 3*A*, lanes 11 and 12), nor PMA induced Rip-1 translocation (Fig. 3*A*, lanes 5 and 6). Both dexamethasone (at 10^{-6} M) (Fig. 3 *A* and *B*, lanes 7 and 8) and hydrocortisone (10^{-6} M), which demonstrated almost complete Rip-1 translocation (Fig. 3*B*, lanes 5 and 6) (activators of GR-II pathway), but not all-*trans*-retinoic acid (Fig. 3*A*, lanes 9 and 10) (activators of retinoic acid receptors and related pathways) induced the nuclear translocation of Rip-1. Furthermore, neither the steroid precursor cholesterol (cyclodextrin conjugated) nor 9-*cis*-retinoic acid induced the nuclear translocation of Rip-1 (data not shown).

***vpr* Mutant Viruses Are Complemented in Trans by Vpr Protein and GR II-Stimulating Steroids.** We examined the effect of glucocorticosteroids on infection of U937 cells with *vpr*$^-$ virus. NL43Δvpr viruses were rendered replication competent by complementation in trans with either Vpr protein, dexamethasone, or hydrocortisone (Fig. 4). The replication of the mutant virus, however, was not affected by either type of retinoic acid or by cholesterol or cyclodextrin. These glucocorticosteroids also increased virus production in U937 cells infected with the wild-type molecular clone NL43.

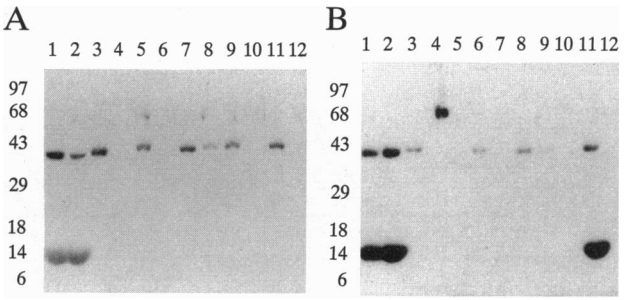

FIG. 3. Steroid induction of Rip-1 translocation. (*A*) Triton X-100 lysates of U937 cells exposed to either Vpr protein (lanes 1 and 2), control supernatants (lanes 3 and 4), PMA (lanes 5 and 6), dexamethasone (lanes 7 and 8), all-*trans*-retinoic acid (lanes 9 and 10), or PBS (lanes 11 and 12). The odd-numbered lanes contain the Triton X-100 crude soluble fraction, and the even-numbered lanes contain the insoluble cytosolic and nuclear fractions. (*B*) Triton X-100 lysates of U937 cells previously exposed to either Vpr protein (lanes 1 and 2), control supernatants (lanes 3 and 4), hydrocortisone (lanes 5 and 6), dexamethasone (lanes 7 and 8), hydrocortisone and mifepristone (lanes 9 and 10), or Vpr protein and mifepristone (lanes 11 and 12). These gels were probed with Vpr protein and a Vpr antibody (as for Fig. 1). The high-molecular-weight background spot was not reproducible in multiple experiments.

GR-II Inhibitors Affect the Vpr-Mediated Effects on Rip-1 and Virus Production. We analyzed the biology of Rip-1 in the presence of mifepristone, a well characterized specific inhibitor of GR-II function (for review, see refs. 24–26, 32). The nuclear translocation of Rip-1 induced by dexamethasone (data not shown) and hydrocortisone was blocked by mifepristone treatment (Fig. 3*B*). Similarly, Vpr-induced translocation of Rip-1 was also blocked by mifepristone (Fig. 3*B*). We examined mifepristone effects on viral replication. The levels of virus produced by infected cells exposed to mifepristone were ≈70% lower than in the untreated cultures. Mifepristone also inhibited the enhancement in virus production seen on addition of Vpr protein or the two steroids (≈80–90% inhibition). Furthermore, the transcomplementation observed for the NL43Δvpr-infected U937 cells by Vpr protein was abolished by mifepristone treatment (Fig. 4 *C* and *G*). The mifepristone inhibitory effects were dose responsive (Fig. 4 *D* and *H*) and present at low concentrations (10^{-6} M).

Vpr and Rip-1 Coimmunoprecipitated with Human GR. These results suggest that Rip-1 may directly associate with the GR-II complex. To examine this hypothesis, we first stimulated U937 cells or cell lysates with vpr. The cells, or lysates, respectively, were then exposed to a reversible (DTSSP) or a nonreversible crosslinking agent (DSS). The samples were immunoprecipitated with an anti-human GR antibody that only binds to the activated GR complex and were examined by immunoblot with anti-GR or by Vpr ligand blot. Fig. 5 shows that the fractions that contain GR also have Vpr and Rip-1. In addition, the fractions that were crosslinked with DSS show that GR, as well as Vpr and Rip-1, is involved in a high-molecular-weight complex. These data demonstrate that Rip-1 is a part of the GR complex after Vpr-induced activation.

DISCUSSION

We identified a cytosolic protein (Rip-1) that interacts with HIV-1 Vpr. A 41-kDa Rip-1 band was detected in both RD

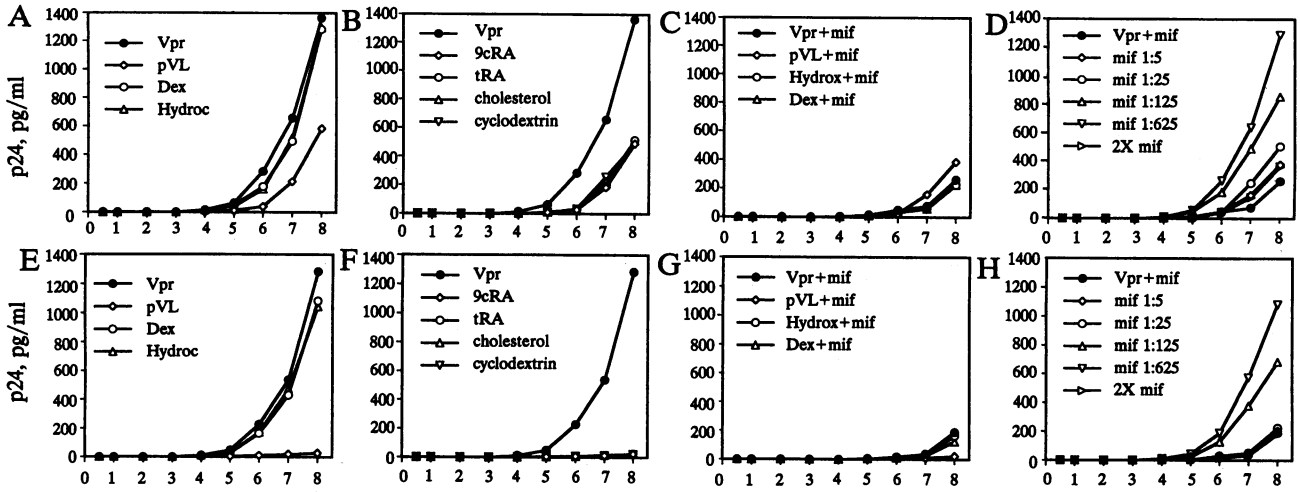

FIG. 4. Viral transcomplementation by Vpr protein and steroids. (*A–D*) U937 cells infected with HIV-1 NL43 (wild type), supplemented with recombinant Vpr protein (Vpr), or control supernatants (pVL), or dexamethasone (10^{-6} M) (Dex), hydrocortisone (10^{-6} M) (Hydroc), 9-*cis*-retinoic acid (9cRA), all-*trans*-retinoic acid (tRA), cholesterol, and cyclodextrin. (*E–H*) Infection profiles for U937 cells infected with HIV-1 NL43Δvpr supplemented with the indicated agents. *A, B, E,* and *F* are in the absence of mifepristone, and *C, D, G,* and *H* are in the presence of mifepristone. The complementation effects observed in *A, B, E,* and *F*, were inhibited by addition of mifepristone (mif) (*C, D, G,* and *H*) to culture medium, at 10^{-6} M.

cells and U937 monocytic cells, as well as in primary lymphocytes and in adherent monocyte/macrophages obtained from a healthy HIV-1 seronegative donor (data not shown); therefore Rip-1 is present in cell lineages that are the primary targets of HIV infection. Rip-1 was not detected, however, in murine NIH 3T3 fibroblastoid cells or in monkey BS-C-1, CV-1, or the related COS cell line (data not shown). Interestingly, these cell lines have been reported to lack endogenous GR (22, 27).

Rip-1 cotranslocated to the nucleus with Vpr, either after exposure of the cells to HIV-1 virus or to exogenous Vpr protein. This nuclear translocation of Rip-1, however, was not induced by an infectious *vpr* mutant virus or by PMA. In addition, the Vpr/Rip-1 nuclear translocation always preceded the accumulation of detectable extracellular virus by 24 hr. This functional correlation of nuclear translocation of the Vpr/Rip-1 complex and virus activation is an interesting aspect of Vpr biology, as the lack of a traditional nuclear localization signal by Vpr appears to be overcome by its interaction with other cytosolic proteins. Ligand-induced cytoplasmic-to-nuclear protein translocation is a characteristic of a limited number of cellular biochemical pathways.

GR-II proteins are members of the ligand-activated transcription factor superfamily of steroid hormone receptors. GRs can act as powerful transactivators (23), involved in the proliferation and further differentiation of committed progenitor cells (28). Resting cells have most of their GR-II in their cytoplasmic compartment, as a protein complex associated with a heat shock protein (hsp) 90 dimer and hsp56 monomer. Upon activation by steroid ligand, the receptor is transformed, and the complex loses the hsp90 dimer and now represents the activated hsp56-associated and hsp70-associated receptor form (27, 29, 30). The activated GR complex represents a less negatively charged DNA-binding protein complex (31). The activated receptor complex shuttles to the nuclear compartment (33, 34), where it binds their specific nucleic acid target sequences (27, 28), inducing specific transcriptional activation. Interestingly, binding sites for GR are found in the HIV long terminal repeat (4, 35, 36). One such glucocortocoid response element site is the TGTTCT GR target sequence, homologous to that which has been defined for the mouse mammary tumor virus long terminal repeat (37). We have observed that Vpr affects transcriptional activation of the HIV long terminal repeat through its ability to directly activate the GR-II pathway (unpublished work). Such a transcriptional regulatory mechanism might serve to unify the observed Vpr effects on cell differentiation and proliferation (11), latency (17), and replication (15).

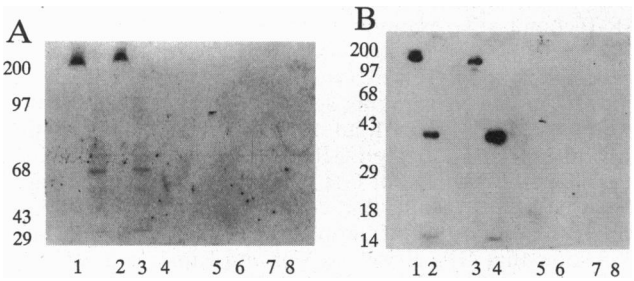

FIG. 5. Coimmunoprecipitation of Vpr/Rip-1 with the human GR-activated complex. U937 cells (lanes 1, 2, 5, and 6) or cell lysates (lanes 3, 4, 7, and 8) were exposed to Vpr at 50 μg/ml (lanes 1–4) or control at 50 μg/ml (lanes 5–8), followed by crosslinking with either DTSSP or DSS. Samples were analyzed by SDS/PAGE under reducing conditions and immunoblotted. Samples were detected with an anti-human GR monoclonal antibody (*A*) or with Vpr–ligand blotting as in Fig. 1 (*B*). (*A* = 10%, *B* = 12% gel.)

The possibility that Rip-1 represents another form of or another member of the GR-II family does not exclude the alternative in which Vpr and/or Rip-1 form a part of the GR transcription complex. It is conceivable that Vpr alters Rip-1 such that it binds to the complex to promote receptor transformation and the subsequent nuclear translocation. A role for Vpr in translocation of the HIV-1 preintegration complex has been recently suggested (38). Vpr through its association with Rip-1 could form the basis of a cellular shuttle for the other members of the complex through its cooperation with nucleocapsid protein. The role of additional Vpr binding proteins is under investigation (42).

The translocation effect of Vpr on Rip-1, as well as the complementation in trans provided by Vpr protein for *vpr* mutant viruses, was closely mimicked by two GR-II-stimulating steroids dexamethasone and hydrocortisone. Specific inhibitors of GR-II translocation and cellular activation have been described (for review, see refs. 24–26). Mifepristone has been reported to reverse the catabolic effect of glucocorticoids in thymocytes *in vitro* (39) by inhibiting the formation and nuclear translocation of the dexamethasone-receptor complexes (29, 39). Mifepristone can also inhibit induction of

Epstein–Barr virus expression in Daudi cells (40), as well as reverse the dexamethasone-induced inhibition of growth in a human cervical-carcinoma cell line (41). We observed that mifepristone could inhibit both the Vpr-induced GR translocation event, as well as the Vpr-induced enhancement of viral replication.

Collectively these data allow speculation into Vpr biology as it relates to the HIV life cycle and viral pathogenesis. Linking Vpr to the GR-mediated transcriptional pathway could establish a link between a viral protein and several pathologies observed in AIDS patients. Glucocorticoids have widespread immunosuppressive effects and are reported to interact with both the NF-κB and FK506 pathways (43, 44), which may have relevance for HIV biology. Glucocorticoids have been reported to induce apoptosis in thymocytes and T cells. Viral-induced apoptosis of lymphocyte precursor populations is implicated in the T-cell depletion, which is a hallmark of HIV infection (for review, see ref. 4). These data are also interesting in view of the several observed pathological features present in some HIV-1-positive patients and also in some patients exhibiting glucocorticoid steroid toxicity. These common symptoms include muscle wasting and susceptibility to fungal infections (45). The GR-II pathway could be exploited in an autocrine fashion by HIV through the action of the *vpr* gene product. Inhibitors of this specific pathway, which include mifepristone (RU486) [11β-(4-dimethylaminophenyl)-17β-hydroxy-17α-(propyl-1-ynyl)-4,9-dien-3-one] among others, may have relevance for the clinical management of HIV infection. Collectively, these studies provide a unified basis for understanding many seemingly disparate activities of Vpr, including nuclear translocation, increased viral replication, cellular differentiation, and inhibition of cell proliferation—as well as providing a context for analyzing the observed transcriptional activational properties of this HIV accessory gene.

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