

Regulation of Human Immunodeficiency Virus Type 1 Gene Transcription by Nuclear Receptors in Human Brain Cells*

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Infection of cells of the central nervous system by the human immunodeficiency virus type-1 (HIV-1) leads to HIV-1-associated neuropathology. Recent studies have demonstrated the importance of long terminal repeat (LTR) binding sites in determining the pathogenicity of HIV. Here we have investigated the presence and the functional role of transcription factors that have the potential to interact, directly or indirectly, with the nuclear receptor-responsive element in the LTR of HIV-1, in different human cell lines of the brain. Cotransfection experiments showed that in oligodendrogloma TC-620 cells, the retinoic acid receptor and the retinoid X receptor activate LTR-driven transcription in the absence of ligand. Addition of all-*trans*- or 9-*cis*-retinoic acid reverses this effect. In contrast, in astrocytoma, neuronal, and microglial cells, no significant effect of the retinoid acid pathway was detected. This retinoid response is mediated by distinct molecular interactions in the lymphotropic LAI and the neurotropic JR-CSF HIV-1 strains. Moreover, retinoid receptors were found to antagonize the chicken ovalbumin upstream promoter transcription factor- as well as the c-JUN-mediated LTR transactivation. Our findings demonstrate the importance of the retinoic acid signaling pathway and of cross-coupling interactions in the repression of HIV-1 LTR gene expression.

The molecular mechanisms controlling HIV-1¹ pathogenesis in the central nervous system are not understood. Tissue macrophages and microglial cells are major target cells and reservoirs for virus during HIV disease in the brain. In addition, infection of neuronal and glial cells also appears to contribute to HIV-1-related pathology (1–4). HIV-1 gene expression is regulated by an interplay of viral and cellular host proteins that interact with a number of binding sites present in the long terminal repeat (LTR) (5). Recent reports highlighted the importance of the U3 region of the LTR in determining the

pathogenicity of HIV-1 (6). Therefore studies concerning the various elements of the modulatory region of the LTR and their interaction with host proteins present in brain cells appear quite crucial.

Transcription factors belonging to the steroid-thyroid-retinoid nuclear receptor superfamily have been shown to interact with the nuclear receptor-responsive element (NRRE) located within the –356/–320 region of the LTR (7–10). The NRRE sequence represents a site for complex regulatory interactions among a variety of hormone receptors, orphan receptors as well as the AP-1 transcription factor. We have recently reported that AP-1 is unable to bind directly to the NRRE sequence and that it interacts with the –247/–222 sequence present in neurotropic HIV-1 strains (10); however, indirect binding of a FOS protein to the NRRE site has been reported (11). Our recent findings indicate that COUP-TF (chicken ovalbumin upstream promoter transcription factor), an orphan member of the hormone receptor superfamily (12, 13), is one of the major species that binds to the NRRE and functions as a potent activator of LTR-driven transcription in brain cells.² A number of studies have described that retinoic acid receptors (RARs) and retinoid X receptors (RXRs), in the presence of their ligand, modulate LTR-driven transcription in non-CNS-derived cells. 9-*cis*-Retinoic acid (RA) activates both RARs and RXRs, whereas all-*trans*-RA only activates RARs (14). Ligand-dependent stimulation of LTR-directed transcription by RAR α and RXR α was observed in choriocarcinoma JEG-3 cells (9) and in CV1 cells, via the NRRE site (8). In the presence of phorbol myristate acetate, transcriptional activity was enhanced in U937 monocytes treated with RA, through a distinct RA and phorbol myristate acetate-responsive element located in the –83/+80 LTR region (15). A negative effect of RA on HIV LTR activity was documented in HeLa and U937 cells, via the NF- κ B element (16). Moreover, various effects of RA on the replication of HIV in different cell types have been reported (17–19). Taken together, these data reveal the importance of the type of target cell in the RAR- and RXR-mediated transcriptional response and point out that distinct molecular mechanisms control the retinoid signaling pathway.

In this report we have investigated the presence of members of the RAR and RXR family in various cell types of the brain. We have further examined the functional effect of RAR- α , RXR- α , and RA treatment on HIV-1 LTR-driven transcription in human neuronal, glial, and microglial cells. Our results show that the nuclear receptors regulate HIV-1 gene expression in a unique manner; unliganded RAR and RXR lead to activation, whereas the addition of RA antagonizes the receptor-mediated activation in oligodendrogloma cells. Moreover, we elucidate the interactive physiological networks of the retinoid receptors RAR and RXR, of the orphan receptor

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¹ The abbreviations used are: HIV-1, human immunodeficiency virus type 1; CNS, central nervous system; LTR, long terminal repeat; RA, retinoic acid; RAR, retinoic acid receptor; RXR, retinoid X receptor; NRRE, nuclear receptor responsive element; tk, thymidine kinase; CAT, chloramphenicol acetyltransferase; PBS, phosphate-buffered saline; COUP-TF, chicken ovalbumin upstream promoter transcription factor.

² Sawaya, B. E., Rohr, O., Aunis, D., and Schaeffer, E. (1996) *J. Biol. Chem.* **271**, in press.

COUP-TF and the transcription factor AP-1, in the regulation of HIV-1 gene expression in various cells of the brain. We show that the retinoid receptors function as repressors of COUP-TF-mediated transactivation and that RXR acts as a repressor of c-JUN-mediated transactivation. Our findings reveal the importance of retinoic acid as well as of cross-coupling interactions in the inhibition of LTR-driven HIV-1 gene transcription.

MATERIALS AND METHODS

Plasmids—To generate LTR(JR-CSF)-CAT, $-283/+20$ LTR-CAT, $-159/+20$ LTR-CAT, the plasmid pSAFYre containing the JR-CSF LTR (gift of Dr. J. Clements; Ref. 20) was digested with *EcoRV* + *Bgl*III, *Eat*I + *Bgl*III, and *Ava*I + *Bgl*III, respectively. The LTR inserts were isolated, blunt-ended, and subcloned in the *Sma*I site of pUC19-CAT0. To generate LTR(LAI)-CAT, $-283/+80$ LTR-CAT and $-159/+80$ LTR-CAT, the plasmid pSV1b-CAT containing the LAI LTR (gift of Dr. N. Israel) was digested with *Bgl*III + *Hind*III, *Eat*I + *Hind*III, and *Ava*I + *Hind*III, respectively. The LTR inserts were blunt-ended and subcloned in the *Sma*I site of pUC19-CAT0. The $-68/+29$ LTR-CAT vector was constructed by subcloning in the *Sma*I site of pUC19-CAT0 the *Bst*NI-*Bst*NI blunt-ended LTR insert. To construct the LTRmut3-CAT vectors, site-directed mutagenesis was performed with the mutant oligonucleotide 3Lmut: 5'-CCAGGGGTAAGATATCCACAAAGCTTTG-3'. To construct the 3L/tk-CAT and 3Lmut/tk-CAT vectors, one copy of the 3L and 3Lmut oligonucleotide was respectively subcloned in the blunt-ended *Sal*I site of pBLCAT2, containing the herpes simplex virus thymidine kinase promoter in front of the CAT gene (gift of C. Kedinger, Unité INSERM 184, Strasbourg, France). The human RAR- α and RXR- α cDNA in the pSG1 vector and the reporter vector containing the RARE DR5G sequence inserted in pBLCAT8+ were a gift of P. Chambon (21). The hc-JUN vector was a gift from C. Quirin-Stricker (Unité INSERM 184, Strasbourg, France), and the COUP-TF vector was a gift from M. J. Tsai (Baylor College of Medicine, Houston, TX).

Cell Culture—Human astrocytoma U373-MG (ATCC HTB17), neuroblastoma SK-N-MC cells (ATCC HTB10), and microglial cells (gift of M. Tardieu; Ref. 22) were grown in Dulbecco's modified Eagle's medium supplemented with 10% charcoal-stripped fetal calf serum and 10 mM HEPES. The medium of neuroblastoma SK-N-MC cells was supplemented with nonessential amino acids. All these cell lines were cultured in the presence of penicillin/streptomycin (100 units/ml). Human oligodendrogloma TC-620 cells (gift of J. E. Merrill; Ref. 23) were grown in Iscove medium, containing 10% nonheat-inactivated fetal calf serum and 1% gentamycin.

Transfections and CAT Assays—Cells (10^6) cultured in medium containing 10% charcoal-stripped fetal calf serum were transfected by the calcium phosphate precipitation method with 1 pmol of plasmid reporter DNA and when indicated with 0.5 pmol of expression vector, as described previously (24). 24 h after transfection, cells were treated for 24 h with $1 \mu\text{M}$ all-*trans*-RA or 9-*cis*-RA in ethanol or with 0.1% ethanol final concentration as a control. Each transfection was done in duplicate and repeated a minimum of three separate times with at least two different plasmid preparations. Cell extracts were prepared 48 h after transfection. CAT assays were performed as described previously (24). Since the efficiency of transfection is different in the different cell lines, different amounts of cell extracts were used, 5, 5, 10, and 20 μg of protein for microglial, astrocytoma, oligodendrogloma, and neuroblastoma cells, respectively.

Western Blot Analysis—Nuclear proteins were extracted from at least 10^8 cells, as described previously (25). Proteins (10 μg) were subjected to SDS-polyacrylamide gel electrophoresis (10% polyacrylamide) and transferred to nitrocellulose paper. Membranes were preincubated with 3% bovine serum albumin in phosphate-buffered saline (PBS) overnight at 4 °C and were probed either with polyclonal antibodies directed against RAR- α (RP α F), RAR- β (RP β F₂), RAR- γ (RP γ F), or with monoclonal antibodies directed against RAR- α (Ab9 α F), RAR- β (Ab8 β F₂), RAR- γ (Ab4 γ F), RXR- α , - β , - γ (1RX-6G12) (1:2000 dilution; gift of P. Chambon) for 90 min at room temperature in PBS-0.1% Tween 20 (PBST). After three washes with PBST, membranes were incubated with peroxidase-labeled anti-rabbit antibody (1:4,000 dilution; Amersham Corp.) for 40 min and washed four times for 30 min in PBST. The signal was visualized by the chemiluminescence protocol (Amersham Corp.).

RESULTS

Pattern of RAR and RXR Expression in Different Human Brain Cells—To analyze the functional effect of retinoid control

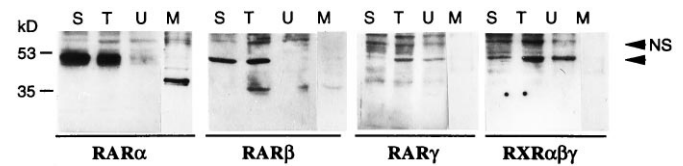


FIG. 1. Western blot analysis of nuclear proteins from human glial, neuronal, and microglial cell lines. Nuclear protein extracts were prepared from neuronal SK-N-MC (lanes S), oligodendrogloma TC-620 (lanes T), astrocytoma U373-MG (lanes U), and microglial (lanes M) cells. Proteins (10 μg) were subjected to an SDS-10% polyacrylamide gel, transferred to a nitrocellulose membrane, and probed with anti-RAR- α , RAR- β , RAR- γ , and RXR- α , - β , - γ monoclonal antibodies (gift of P. Chambon). The signal was visualized using the enhanced chemiluminescence system (Amersham Corp.). The upper band (NS) in each panel corresponds to a nonspecific immunoreaction. Arrowhead indicates the endogenous retinoic acid receptors. Lower molecular weight reactive components were found in microglial cells. The position of the prestained molecular mass markers (Bio-Rad) is indicated in kilodaltons (kDa).

of HIV in different human brain cells, we first examined the presence of endogenous retinoic acid receptors RAR- α , - β , or - γ and RXR- α , - β , or - γ . Western blot analysis was performed with nuclear protein extracts from human oligodendrogloma TC-620, astrocytoma U373-MG, neuroblastoma SK-N-MC, and microglial cells using a set of monoclonal antibodies directed specifically against human RAR- α , RAR- β , RAR- γ , and RXR- α , - β , - γ (26, 27). As shown in Fig. 1, the expected RAR- α species with an apparent molecular mass of 51 kilodalton (kDa) were detected with antibodies Ab9 α F; RAR- α was expressed at a high level in SK-N-MC and TC-620 cells. With microglial proteins, a 40-kDa band, specifically detected with antibodies Ab9 α F, could correspond to a truncated RAR- α resulting either from alternative splicing events or artifactual proteolysis. RAR- β of expected molecular mass 51 kDa was recognized with antibodies Ab8 β (F)₂, only with SK-N-MC and TC-620 proteins. Antibodies Ab4 γ F allowed the detection, only in glial cells, of RAR- γ of expected molecular mass 51 kDa. Antibodies 1RX6G12 specific for RXR- α , - β , and - γ detected RXR species in glial and neuronal cells but not in microglial cells. Similar results were obtained when the blots were probed with polyclonal antibodies, instead of monoclonal antibodies. These results point out the cell type-specific expression of RARs and RXRs in brain cells. While in TC-620 cells, all RARs and RXR species were expressed at a high level, in U373-MG cells, the level of RAR- α , - γ and RXRs was considerably lower. In SK-N-MC cells, all species were expressed, except RAR- γ . Surprisingly, in microglial cells, no typical RAR or RXR protein could be detected.

Functional Effect of Retinoic Acid Receptors on Reporter Gene Expression in Brain Cells—We first tested the contributions of RAR and RXR to ligand-dependent transcription from a promoter containing a retinoic acid response element (RARE) linked to the herpes simplex thymidine kinase promoter and the CAT gene. The response element consists of two direct repeat half-sites of the GGGTCA sequence separated by a 5-base pair spacer; it binds efficiently RAR or RXR homodimers and RAR/RXR heterodimers (21). Each brain cell line was transfected with the RARE-tk-CAT vector, and CAT activities were determined. In the absence of transfected receptors, all-*trans*-RA elicited a 4–5-fold transcriptional response in glial cells, confirming the presence of functional endogenous receptors (Fig. 2). 9-*cis*-RA elicited a 2.4-fold stimulation in TC-620 cells, indicating the presence of functional RXR receptors. Overexpression of RAR- α or RXR- α increased the transcriptional response to all-*trans*- or 9-*cis*-RA, respectively, in all cell lines, with varying intensities depending on the cell line (Fig. 2). These results are consistent with a number of studies in

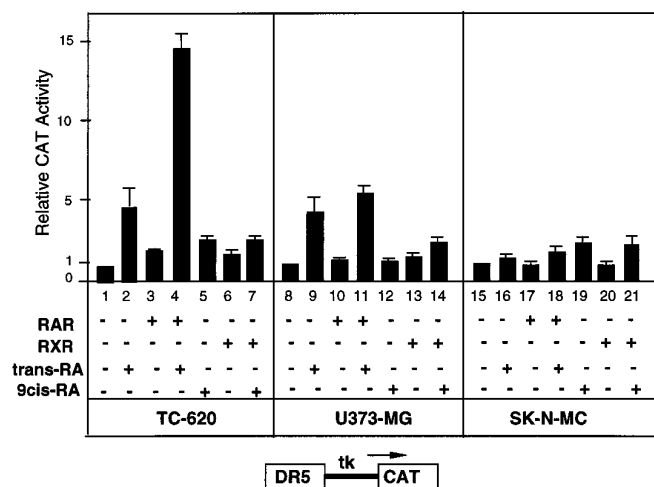


FIG. 2. Effect of all-*trans*- and 9-*cis*-RA on transcription from a promoter containing a DR5 response element. Transient expression experiments were performed in human oligodendrogloma TC-620, astrocytoma U373-MG, and neuroblastoma SK-N-MC cells. A reporter vector containing the DR5G retinoic acid response element linked to the herpes simplex thymidine kinase (tk) promoter and the CAT gene (21) was cotransfected with expression vectors for RAR- α or RXR- α as indicated. 24 h after transfection, cells were exposed to 1 μ M of either all-*trans*-RA or 9-*cis*-RA as indicated. Cell extracts were prepared 48 h after transfection, and CAT assays were performed. Results are the average of at least three independent experiments done in duplicate.

many other cell types indicating that RAR and RXR behave as ligand-inducible transcriptional activators (8, 28, 29).

Ligand-independent Activation of LTR-directed Gene Transcription by RAR- α or RXR- α in Brain Cells—To analyze the regulation of HIV-1 gene expression by RAR, RXR, and retinoic acid in different brain cells, we performed cotransfection experiments with an expression vector for RAR- α or RXR- α and a HIV-1 LTR-CAT reporter construct. We used two distinct LTR-CAT constructs containing the LTR of either the lymphotropic LAI strain or the neurotropic JR-CSF strain (30). After 24 h, cells were either treated with 10⁻⁶ M all-*trans*- or 9-*cis*-RA or left untreated. After further 24 h, cells were harvested and CAT activities were determined. In TC-620 cells incubated in the absence of RA, overexpression of RXR- α and RAR- α resulted in a respective 3- and 4-fold transcriptional activation (Fig. 3). Surprisingly, this stimulation was inhibited by the presence of RA. Similar effects were observed with both types LAI and JR-CSF LTR. This RA-mediated inhibition contrasts with the RA-induced activation observed with the RARE-tk-CAT vector in the same cells, suggesting that the configuration of the retinoic response element in the HIV-1 LTR could be responsible for this effect. In contrast, in U373-MG, SK-N-MC, and microglial cells, retinoic acid receptors, and RA were unable to significantly modify HIV-1 CAT activity (Fig. 3). The effect of RA concentration on RA-mediated inhibition of LTR activity was tested in TC-620 cells. Maximal inhibition by RA was achieved at 10⁻⁸ M and half-maximal inhibition occurred at 10⁻¹⁰ M (Fig. 4).

Retinoic Acid Receptors Act on the NRRE and on Downstream-located Sites of the HIV-1 LTR in TC-620 Cells—Previous studies performed in non-CNS-derived cells have shown that RAR and RXR stimulate transcription of the HIV-1 LTR via the NRRE site (8, 9). Other investigators (16) have shown that RA inhibits transactivation of the HIV-1 LTR via the NF- κ B element in HeLa cells. To identify the region of the LTR responsible for the retinoic response in TC-620 cells, we performed transfection experiments using LTR-CAT vectors containing progressive 5' deletions of the LTR LAI and JR-CSF region (Fig. 5). Deletion of the NRRE site up to position -283

(constructs 2) reduced the RAR- and RXR-mediated transcriptional activation of LTR LAI by 40% and did not modify the activation of LTR JR-CSF. Removal of the AP-1 site present in the LTR JR-CSF (construct 3) resulted in a 60% decrease of CAT activity. Further deletion of the NF- κ B sites up to position -68 (construct 4) abolished the transcriptional stimulation (Fig. 5). These results point out the difference in the molecular interactions that control LTR LAI and LTR JR-CSF gene transcription. They indicate that the NRRE site of LTR JR-CSF is not involved in the RAR-mediated effect; cross-coupling interactions, especially with the AP-1 element, are likely to contribute for the most part to the retinoid effect. In contrast to LTR JR-CSF, the NRRE site of LTR LAI is involved to some extent in the RAR-mediated activation, together with downstream-located elements, such as NF- κ B. However, the precise nature of the complex molecular interactions remains to be determined.

Regulation of HIV-1 (JR-CSF) LTR-driven Expression by Transcription Factors Acting on the NRRE Site in Brain Cells—To examine the effect of heterodimerization of nuclear receptors on LTR-driven gene transcription, we cotransfected TC-620 cells with RAR- α and RXR- α vectors in equal amounts. Surprisingly, the results showed that, in contrast with RAR or RXR homodimers, RAR/RXR heterodimers possess no ability to enhance transcription (Fig. 6).

Previous reports have described that RAR or RXR are able to form heterodimers with the orphan nuclear receptor COUP-TF (31, 32). We have recently shown that COUP-TF, present in different brain cells, interacts with the NRRE sequence (10) and functions as a potent activator of LTR-directed HIV-1 gene expression in TC-620 cells (Fig. 6).² COUP-TF has been described as a repressor of the retinoid response of the HIV-1 LTR in CV-1 cells (8). We therefore investigated whether COUP-TF could antagonize RAR- or RXR-mediated induction from the neurotropic JR-CSF LTR. Cotransfection experiments performed in TC-620 cells showed that COUP-TF did not repress the retinoid action; in contrast, the retinoid receptors functioned as repressors of COUP-TF-induced activation and reduced transcription to a level similar to that obtained with the receptors alone (Fig. 6).

Nuclear receptors are known to modulate gene expression by acting as transrepressors of the transcription factor AP-1 (JUN-JUN, or JUN-FOS) activity (for review, see Ref. 33). We have reported previously that c-JUN and COUP-TF were able to stimulate 7- and 10-fold, respectively, the CAT activity of the LTR(JR-CSF)-CAT vector (Ref. 10; Fig. 6).² We therefore investigated by cotransfection experiments whether the combined action of different nuclear receptors and c-JUN was able to modulate HIV-1 gene transcription. When both COUP-TF and c-JUN expression vectors were cotransfected together, c-JUN did not significantly alter COUP-TF-mediated stimulation. Interestingly, overexpression of both RXR and c-JUN proteins resulted in a drastic decrease of the c-JUN-induced stimulation. In contrast, RAR was unable to antagonize the positive c-JUN response (Fig. 6). These results reveal the importance of RXR in repressing the c-JUN-mediated transactivation of the HIV-1 LTR.

To examine whether cross-coupling interactions between nuclear receptors and AP-1 occurred on the NRRE site, we first performed transfection experiments using a reporter vector containing the NRRE sequence linked to the thymidine kinase promoter and the CAT gene. Interestingly, this 3L-tk-CAT reporter vector led to a 4-fold increase in CAT activity in the presence of the COUP-TF expression vector and to a 5-fold increase in the presence of a c-JUN expression vector (Fig. 7, lanes 12 and 13). Overexpression of both c-JUN and COUP-TF

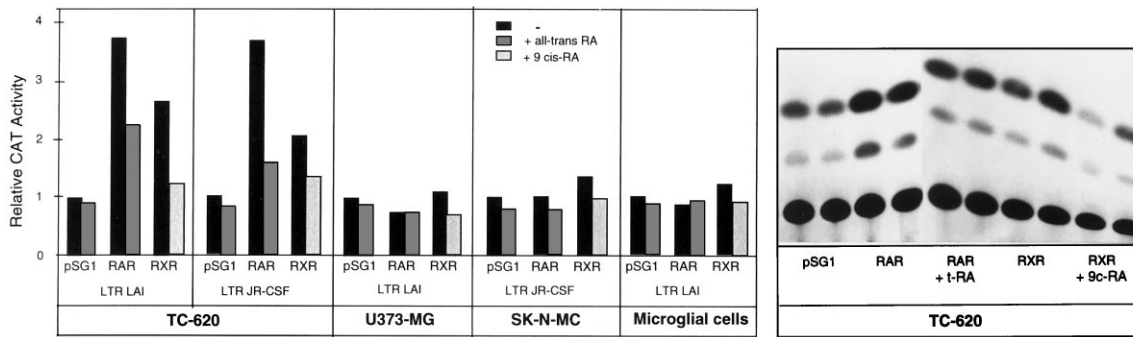


FIG. 3. **Ligand-independent activation of HIV-1 LTR by RAR- α and RXR- α .** Expression vectors encoding RAR- α or RXR- α (0.5 pmol) or the parental pSG1 vector were cotransfected into the human brain cell lines with the indicated HIV-1 LTR-CAT reporter constructs (1 pmol). The LTR LAI extends from position -489 to +80. The LTR JR-CSF contains the -421 to +20 region. 24 h after transfection, cells were incubated for further 24 h in the presence (lanes +) or the absence (lanes -) of either all-*trans*-RA (lanes pSG1 and RAR) or 9-*cis*-RA (lanes RXR). CAT activities were determined as described under "Materials and Methods." *Top*, histograms show the relative CAT activities calculated from the value of the pSG1 control set at 1. *Right panel*, autoradiograms of one typical CAT assay obtained with TC-620 cells.

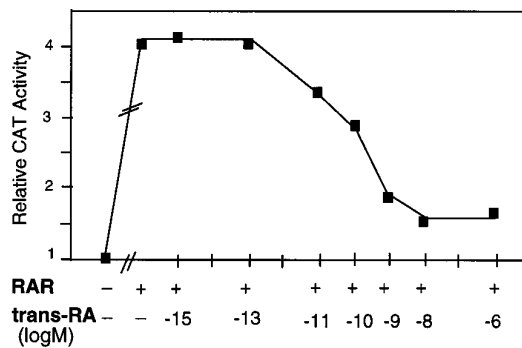


FIG. 4. **Retinoic acid dose response of HIV-1 LTR-CAT activation by RAR.** HIV-1 LTR(JR-CSF)-CAT (1 pmol) and RAR- α expression vector (0.5 pmol) were cotransfected into TC-620 cells. Cells were treated with all-*trans*-RA for 24 h, and CAT activities were determined as described in Fig. 2. Results represent the average of four experiments done in duplicate.

led to a 7-fold increase in CAT activity (lane 16). As a control, the activity of the control 3Lmut-tk-CAT vector, containing a mutant NRRE site unable to bind COUP-TF, remained unchanged (lanes 17–24). Since we recently demonstrated that AP-1 is unable to directly bind to the NRRE sequence (10), this result suggests that AP-1 is able to interact, directly or indirectly, with COUP-TF bound to the NRRE site.

Surprisingly, in TC-620 cells, the NRRE site was unable to confer RAR or RXR responsiveness on the tk promoter (lanes 10 and 11), in contrast to results reported in F9 cells (7). However, cotransfection of RAR or RXR and the c-JUN expression vector strongly decreased the c-JUN-induced stimulation (lanes 14 and 15). Since c-JUN interacts indirectly with the NRRE site, this result reveals that c-JUN is able to modulate transcription via the NRRE site by interacting, directly or indirectly, with COUP-TF, RAR, or RXR.

We recently described that overexpression of c-JUN increased the CAT activity of the 5N-tk-CAT vector, containing the AP-1 binding site spanning the -247/-222 region of the JR-CSF LTR (10). It was interesting to test whether, vice versa, RXR was able to modulate the positive effect of c-JUN acting on its DNA binding site. Overexpression of RXR and c-JUN resulted in a 50% decrease of c-JUN-induced stimulation (results not shown), indicating that cross-coupling interactions between RXR and c-JUN modulate transcription from the 5N-tk promoter. Taken together, these results suggest that, within the JR-CSF LTR, RXR and c-JUN modulate transcription by acting either on the NRRE or on the AP-1 site.

DISCUSSION

In this report we have investigated the regulation of HIV-1 gene transcription in human brain cells by transcription factors that interact, directly or indirectly, with the nuclear receptor response element (NRRE), spanning the -356/-320 region of the lymphotropic LAI and the neurotropic JR-CSF LTR.

Regulation of HIV-1 LTR-directed Gene Transcription by RAR, RXR, and RA—We have first focused our studies on nuclear receptors belonging to the RAR and RXR families. Our results show that different members of the RAR and RXR families are present in human glial and neuronal cells. Surprisingly, no typical member of the retinoid family could be detected in human microglial cells, which represent the primary target of HIV-1 infection in brain. Since these results were obtained in a microglial cell line, it would be interesting to examine the existence of these receptors using primary cultured microglial cells.

Nuclear receptors are known to mediate both positive and negative effects on promoter activity in response to ligand binding (34). A number of reports have described the use of retinoids to alter HIV-1 replication in certain cell types. Retinoic acid exerts various effects on the replication of HIV, which depend on the type of target cell and the time of treatment (17–19).

Our transient expression data indicate that in different brain cells RAR- α and RXR- α are able, in response to ligand binding, to activate transcription of a thymidine kinase promoter driven by a retinoic acid response element. However, these receptors are unable, in the presence or absence of their ligand, to affect HIV-1 LTR-driven gene expression in astrocytoma, neuronal, and microglial cells. In contrast, in oligodendrogloma TC-620 cells, RAR- α and RXR- α function as activators of HIV-1 gene transcription in the absence of ligand binding. Interestingly, in the presence of all-*trans*- or 9-*cis*-retinoic acid, the stimulation mediated by unliganded RAR or RXR is almost completely reversed. A similar negative effect of RA on HIV-1 LTR activity was recently described in HeLa cells and the U937 monocyte cell line (16). In contrast, a RA-dependent stimulation of HIV-1 gene transcription by RAR and RXR was reported in choriocarcinoma JEG-3 cells and in CV1 cells (8, 9). These distinct transcriptional effects may be accounted for by the existence of recently described cell-type specific co-activators or co-repressors (35, 36).

Several mechanisms of negative regulation by nuclear receptors have been reported (33, 34). One mechanism has been described for thyroid hormone receptors (T3R); thyroid hormone (T3) inhibits stimulation mediated by unliganded T3R, in

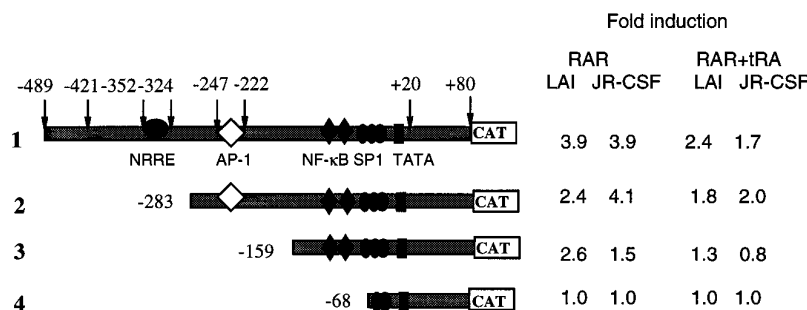


FIG. 5. Delineation of RA-responsive elements by deletion analysis of the HIV-1 LAI or JR-CSF LTR. *Right panel*, plasmid constructs of the HIV-1 LTRs used in transient expression assays. The LTR LAI (−489/+80) and LTR JR-CSF (−421/+20) were 5′-end-deleted. The Ap-1 site is not present in LTR LAI. *Left panel*, fold stimulation in CAT activity obtained with TC-620 cells cotransfected with the LTR-CAT constructs and the RAR- α expression vector, followed by all-*trans*-RA treatment 24 h after transfection.

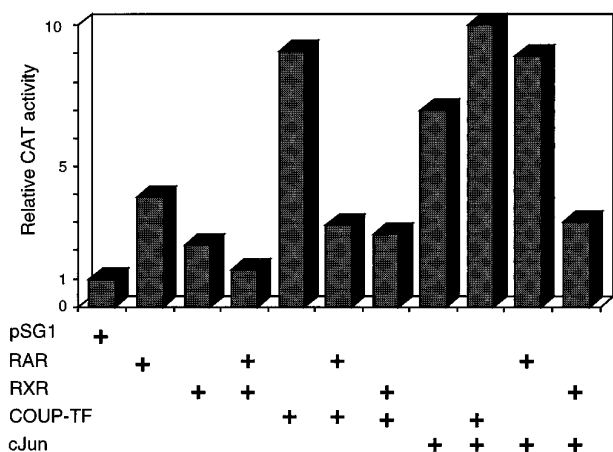


FIG. 6. Regulation of HIV-1 LTR-driven transcription by nuclear receptors in TC-620 cells. Transient expression experiments were performed in TC-620 cells. The reporter vector HIV-1 LTR(JR-CSF)-CAT (1 pmol) was cotransfected with either the parental pSG1 plasmid or vectors (0.5 pmol) expressing transcription factors as indicated. Cell extracts were prepared 48 h after transfection, and CAT activities were determined. Values correspond to an average of at least three independent experiments done in duplicate. The standard deviation did not exceed 20%.

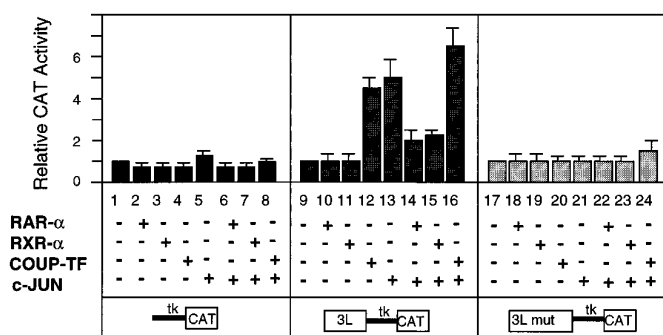


FIG. 7. Transactivation of reporter genes containing the NRRE site by coexpressed RAR- α , RXR- α , COUP-TF, or c-JUN. CAT assays were performed with extracts of TC-620 cells that were cotransfected with the indicated pBL-CAT2 reporter vectors (shown on bottom) and expression vectors (on the left). Oligonucleotides 3L and 3Lmut correspond to the wild-type and mutant NRRE sequence, respectively. CAT activities are expressed relative to the activity of each reporter vector set at 1. They correspond to the average of at least three independent experiments done in duplicate.

the LTR of Rous sarcoma virus and in the LTR of HIV-1. T3R has been shown to interact with several sites in the HIV-1 proximal promoter spanning the NF- κ B and the Sp1 elements (37), although other investigators found that T3R binds to a single site overlapping the Sp1 sequence (38). A second mechanism involves blocking a positive transcription factor such as

AP-1; it appears that DNA binding by the receptor may not be sufficient or even required for ligand-dependent repression and that an interplay with additional factors may be involved.

Our findings reveal that, depending on the LTR sequence, both mechanisms are likely to be involved in the RAR and RXR regulation of HIV-1 gene transcription in TC-620 cells. In the lymphotropic HIV-1 LAI strain, the NRRE site plays a major role in RAR- and RXR-mediated transcriptional stimulation. Similarly, the NRRE site was found to be responsible for the ligand-dependent activation mediated by RAR and RXR in JEG-3 (9) and in CV1 cells (8). When the NRRE sequence was deleted, downstream-located elements, such as NF- κ B, are able, to some extent, to mediate the RAR and RXR action in TC-620 cells. Similarly, the NF- κ B element was shown to be responsible for the negative effect of RA on HIV LTR activity in HeLa and U937 cells (16). In contrast, the NRRE site in the LTR of the neurotropic JR-CSF strain is not indispensable for the retinoid effect, since removal of the NRRE region did not affect transcription. The retinoid action can be mediated by downstream-located elements, such as the −247/−222 AP-1 region, recently described in the LTR JR-CSF (10) and, to a lesser extent, the NF- κ B region. Similarly, distinct mechanisms, depending on the LTR sequence, were found to govern COUP-TF-induced stimulation in TC-620 cells.² These data show how sequence variations in regulatory sites within the LTR modify the binding properties of transcription factors and further demonstrate the flexibility of the interactions between various elements of the LTR.

Regulation of HIV-1 JR-CSF LTR-directed Gene Transcription by RAR and RXR in the Presence of COUP-TF or AP-1—Multiple nuclear receptors bind to the NRRE and modulate HIV-1 LTR-driven transcription in non-CNS-derived cells (9). We have previously shown that the orphan nuclear receptor COUP-TF is expressed at a high level in brain cells and leads to a dramatic transcriptional stimulation in oligodendrogloma TC-620 cells.² In contrast to results described in CV1 cells, where COUP-TF had no action by itself and repressed the retinoid response (8), our data reveal that either RAR or RXR is able to dramatically inhibit the COUP-TF function. Moreover, high levels of RXR expression repress RAR activity, which suggests that the transcriptional activity of the LTR may be modulated by the relative amount of RXR present in TC-620 cells. Thus regulation of LTR-driven transcription may depend on the actual cell concentration of RARs, RXRs, COUP-TF, as well as of cell-specific transcriptional intermediary factors, which are responsible for coupling the transcription factors to the transcription machinery.

In addition, our findings reveal that the nuclear receptor signaling pathway, by interfering with the AP-1 pathway, is able to inhibit HIV-1 gene expression. We have described previously that the transcription factor AP-1, composed of the JUN

and FOS proteins, stimulates transcription from the LTR(JR-CSF) promoter in TC-620 cells, by acting on the -247/-222 binding site (10). Here our data further reveal the existence of direct or indirect interactions between the c-JUN component of AP-1 and nuclear receptors bound to the upstream-located NRRE site. Vice versa, cross-coupling interactions are likely to occur between RXR and c-JUN acting on the AP-1 site. These findings may explain the antagonism between RXR and c-JUN, which leads to a strong inhibition of the c-JUN response. In contrast RAR is not able to antagonize the positive action of c-JUN. A number of studies have demonstrated the interplay between the regulatory circuits stimulated by AP-1 and those activated by nuclear receptors. Inhibition of AP-1 activity by RARs was reported in non-CNS-derived cells, and RXRs were described to inhibit AP-1 binding to its TRE site *in vitro*. Vice versa, AP-1 was shown to antagonize the activity of several hormone receptors through protein-protein interactions (for review, see Ref. 33). Interestingly, while RAR-mediated AP-1 transrepression has been described to be ligand-dependent, our findings reveal a ligand-independent effect. The molecular mechanisms underlying AP-1 transrepression by RAR or RXR remain elusive; however, the concept has emerged that AP-1 and nuclear receptors do not interact directly but act indirectly through transcriptional mediators (for review, see Ref. 33).

Our data reveal the complexity of the protein-protein interactions between nuclear receptors and AP-1 as well as the diversity of their interactions with different LTRs. Since the AP-1 transcription factor is composed of different members of the JUN and FOS family, it would be interesting to examine whether the composition of the AP-1 complex is critical for RXR-mediated repression and for overall modulation of HIV-1 gene transcription. In conclusion, our data demonstrate the importance of the retinoid receptor pathway in the inhibition of HIV-1 gene transcription in TC-620 cells. It is noteworthy that the positive effect of RAR or RXR is inhibited by the presence of retinoic acid. Moreover, while RAR contributes to repress the positive COUP-TF response, RXR, by antagonizing the positive action of distinct transcription factors, such as RAR, COUP-TF, and AP-1, is a potent negative regulator of HIV-1 gene transcription.

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