Inhibition of NF-κB and HIV-1 Long Terminal Repeat Transcriptional Activation by Inducible Nitric Oxide Synthase 2 Activity*

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In the human lymphoblastoid T cell line JJhan-5.1, stably transfected with a human immunodeficiency virus-1 long terminal repeat luciferase vector, the level of luciferase activity is dependent on activation of the nuclear factor κB (NF-κB) transcription factor. Tumor necrosis factor-induced luciferase activity was not modified in JJhan-5.1 cells co-cultivated with murine adenocarcinoma EMT-6 cells but was strongly decreased when nitric oxide (NO) synthase 2 expression was induced in these cells. Two NO synthase inhibitors counteracted this inhibitory effect. Tumor necrosis factor-α binding to JJhan-5.1 cells was not modified after incubation with EMT-6 cells. Viability and protein synthesis in JJhan-5.1 cells were also unchanged. Induction of NF-κB DNA binding activity was inhibited when EMT-6 cells expressed NO synthase 2 activity. Amionoguanidine, which completely abolished nitrite production, prevented this inhibition. NF-κB activation was also strongly inhibited by S-nitrosoglutathione but was marginally affected by N-(2-aminoethyl)-N'-(2-hydroxy-2-nitrosohydrazino)-1,2-ethylenediamine. Taken together, these results indicated that NO-related species, released by EMT-6 effector cells and probably different from NO itself, inhibited NF-κB activation in human lymphoblastoid target cells. Consequently, transcriptional activity of a long terminal repeat-driven luciferase gene construct was markedly diminished.

Nitrergic oxide is a diffusible, cell-permeable reactive molecule synthesized by three P-450-type NO synthase (NOS) isoenzymes, NOS 1, 2, and 3. NOS 1 and 3, usually constitutive, are transiently activated by Ca²⁺/calmodulin binding and were initially characterized in neuronal and vascular endothelial cells, respectively (reviewed in Refs. 1 and 2). The cytokine-inducible NOS 2 can be expressed in a wide range of cell types and tissues and, once induced, generates a sustained flux of NO that has cytotoxic consequences for tumor cells and pathogens (3, 4). For instance, nitric oxide and related reactive nitrogen species have antiviral properties, most frequently shown for DNA viruses but also, in vivo, for the RNA Cosaxkje B virus and the Friend leukemia murine retrovirus (5–8). At a molecular level, the antiviral mechanisms of nitrogen oxides are only partially understood. Viral DNA synthesis requires efficient deoxynucleotide synthesis. It has been proposed that inhibition of viral ribonucleotide reductase might partially explain the inhibition of DNA virus replication (6, 7). This mechanism might also be relevant to retroviruses, as reverse transcriptase and ribonucleotide reductase inhibitors synergize against HIV infection (9). NO can also affect the viral life cycle by altering intracellular signals required for viral replication, including transcription factors. The transcription factor Zta, which mediates the switch from latent to lytic infection in Epstein-Barr virus-infected cells, is down-regulated by NO (10). Two C-nitroso compounds have also been shown to inhibit HIV-1 infectivity by ejecting zinc from a zinc finger transcription factor (11). The importance of NO production in HIV-1 infection has been already established. It has been proposed that activation of neuronal NOS 1 activity by the gp120 envelope protein might explain the neurotoxicity of the virus (12). There is also evidence that gp120 and gp41 protein determinants can induce NOS 2 activity in human glial cultures and that retroviral infection induces NOS 2 expression in human monocyes (13, 14). These results have suggested a detrimental role for NO in HIV-associated pathology. However, analysis of nitrite serum levels in HIV-1-infected, seronegative children has led to the conclusion that NO might be involved in limiting the infection (15). Moreover, the onset of functional NOS 2 expression in HIV-infected monocyte cultures was correlated with a sudden drop in reverse transcriptase activity (13), a result that could be interpreted as a negative effect of NO on retroviral replication. The long terminal repeat (LTR) sequence of HIV-1 contains positive and negative transcriptional regulatory elements that control the expression of the integrated proviral genome (reviewed in Ref. 16). Among these, two NF-κB binding sites constitute the viral enhancer (16, 17). NF-κB is a p65(RelA)/p50 heterodimeric transcription factor belonging to the NF-κB/Rel family, remained inactive in the cytoplasm by association with inhibitory molecules (I-κB) (reviewed in Ref. 18). Phosphorylation of I-κB and, in most cases, subsequent proteolysis enables translocation of NF-κB to the nucleus, where its binding to cognate DNA sequences tranregulates gene expression. In different models, NF-κB activation has been shown to be inhibited by nitrogen oxides (19–23), although in others, including human peripheral blood mononuclear cells, NO activated the NF-κB signaling pathway (24–26). Since viral components are able to trigger NO production in host cells, it was...
of interest to determine whether NO might influence the NF-κB-dependent transcriptional activity of the HIV-1 LTR, either positively or negatively. Bioactive species derived from NOS activities include NO, N2O3, nitrosothiols, dinitrosyl-iron complexes and peroxynitrite, and probably other species as well, each exhibiting a specific reactivity (1). This complexity cannot be reproduced by using chemical NO precursors, although these molecules have been used extensively in previous studies and have therefore contributed most of our current knowledge about NF-κB modulation by nitrogen oxides. The role of endogenously produced NO has been examined in a few models, involving apparently constitutive NOS 1 or 3 activities (21, 23, 27). So far, the effect of NOS 2 products on NF-κB induction in target cells has not been evaluated.

In the present work, we used murine EMT-6 cells activated for NOS 2 expression as effector cells capable of producing physiological concentrations of NO-related species over a prolonged period (28), to test the hypothesis that NO might modulate HIV-1 LTR activity through NF-κB function. We chose a human lymphoblastoid target cell line transfected with a luciferase reporter gene controlled by the HIV-1 LTR promoter sequence.

**EXPERIMENTAL PROCEDURES**

**Cell Cultures and Reagents**—The Jhan-5.1 clone was derived from the J. Jhan cell line (itself derived from Jurkat cells) stably co-transfected with a LTR-Luc plasmid carrying the luciferase reporter gene under the control of the USR (Bagli)/HindIII fragment of HIV-1 LTR and the pSV2-TK-Neo vector carrying the G418 resistance gene under the control of the SV40 early promoter (29). Jhan-5.1 and J. Jhan human lymphoblastoid T cell lines, as well as the EMT-6 murine mammary adenocarcinoma cell line (28), were grown in RPMI 1640 medium (Life Technologies, Inc., Cergy-Pontoise, France), supplemented with antibiotics, 5% heat-inactivated fetal calf serum, 300 mg/ml l-glutamine, and 25 mM HEPES, pH 7.4. G418 (Life Technologies, Inc.) was added in Jhan-5.1 cultures at 400 μg/ml and removed 24 h before experiments. Murine recombinant IFN-γ (specific activity, 1 × 10^6 units/mg) was provided by Dr. Adolf (Ernst-Boehringer Institut für Arzneimittel Forschung, Vienna, Austria). Human recombinant TNF-α (specific activity, 1.6 × 10^6 units/mg) was a gift from Dr. Boussau (Rhône-Poulenc Rorer, Vitry-sur-Seine, France). IL-1α and IL-1β (specific activity, 1.6 × 10^6 units/mg) was provided by Dr. Baglioni (San Giovanni e Paolo, Rome). Human recombinant TNF-α (specific activity, 1.2 MBq/ml) was a gift from Dr. Bousseau (Rhône-Poulenc Rorer) and centrifuged for 10 min at 10,000 × g at 4 °C. The cell pellet was carefully collected and lysed with 0.5 mL of 0.1% Triton X-100. Radioactivity in the lysate and in the aqueous phase was determined by liquid scintillation counting. Calculation of the TNF binding dissociation constant (Kd) and the number of binding sites was done using Multifit 2.0 software (Databro scrambler, Cambridge, UK).

**Nitrite Assay**—Nitrite concentrations in cell culture supernatants were measured with the Griess reagent, as described previously (32).

**Cell Viability Assay**—The viability of Jhan-5.1 cells exposed to stimulated EMT-6 cells for 4 h was assayed, using the trypan blue exclusion test.

**RESULTS**

Jhan-5.1 cells that have been stably transfected with an HIV-1 LTR-driven luciferase reporter gene express constitutive luciferase activity at a low level, and this activity is considerably enhanced by NF-κB activators such as TNF-α. To explore a possible effect of nitrogen oxides on HIV-1 LTR activation, Jhan-5.1 cells were exposed to NO produced enzymatically by murine EMT-6 cells, as described above. They were adjusted to 20 × 10^6 cells/ml and incubated for 6 h at 4 °C in 100 μl of culture medium containing 0.1–5 mM human 125I-labeled TNF-α. Another set of similar samples was incubated with 40-fold excess of cold TNF-α to determine the nonspecific binding. The incubation mixture was then transferred over 400 μl of a silicon oil phase (Rhosoril, Rhône Poulenc Rorer) and centrifuged for 10 min at 10,000 × g. The cell pellet was carefully collected and lysed with 0.5 mL of 0.1% Triton X-100. Radioactivity in the lysate and in the aqueous phase was determined by liquid scintillation counting. Calculation of the TNF binding dissociation constant (Kd) and the number of binding sites was done using Multifit 2.0 software (Databro scrambler, Cambridge, UK).
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TABLE I

Inhibition of TNF-induced luciferase expression by EMT-6 cells expressing NOS 2 activity

Results are mean ± S.E. of (n) independent experiments.

| Culture conditions | Inhibitor | Luciferase activity | Nitrite | (n) |
|--------------------|-----------|--------------------|--------|-----|
|                    |           | −TNF               | +TNF   |      |
| Medium             | None      | 48 ± 4 (100)       | 1095 ± 85 (100) | <1 | 7 |
| EMT-6 cells        | AG        | 46 ± 2 (96)        | 983 ± 92 (90)   | ND | 2 |
|                    | l-NAME    | 49 ± 1 (102)       | 1060 ± 31 (97)  | ND | 3 |
|                    | Unstimulated | None | 47 ± 4 (98)       | 942 ± 65 (86)   | <1 | 7 |
|                    | Stimulated | None | 44 ± 6 (91)       | 303 ± 23 (27)   | 12.2 ± 1.4 | 7 |
|                    | AG        | ND                 | 809 ± 96 (74)   | <1 | 2 |
|                    | l-NAME    | ND                 | 661 ± 74 (60)   | 4.0 ± 1.3 | 3 |

- JJhan-5.1 cells were cultured in medium alone or co-cultured with EMT-6 cells that have been previously stimulated, or not, for NOS 2 expression. After 4 h, JJhan-5.1 cells were carefully harvested and incubated for 5 h with TNF-α for luciferase induction (+TNF), or left untreated (−TNF).
- NOS inhibitors aminoguanidine (AG) or l-NAME were present at a concentration of 2 mM, only during the co-culture period.
- Luciferase activity was measured in JJhan-5.1 cells, as described under “Experimental Procedures,” and expressed as luminescence counts or as the percentage of the activity in respective control JJhan-5.1 cells cultured alone. Mean luciferase background level, 8 counts.
- NO production by EMT-6 cells was reflected by the concentration of nitrite accumulated in co-culture supernatants.
- ND, not determined.
- Statistically different from JJhan-5.1 cells cultured in medium alone.
- Statistically different from JJhan-5.1 cells co-cultured with stimulated EMT-6 cells, by paired Student’s t test.

5.1 cells cultured alone (mean ± S.E., n = 4).

In the absence of NOS 2 induction, the constitutive and TNF-induced luciferase activities were not significantly modified when JJhan-5.1 cells were co-cultured for 4 h with EMT-6 cells (Table I). The low constitutive luciferase activity of JJhan-5.1 cells was also unchanged upon co-culture with stimulated EMT-6 cells. In these experiments, nitrite production, taken as an index of NO 2 activity in EMT-6 cells, was as high as in macrophage cultures, ranging from 2 to 4 μM per h. Therefore, in this model, a sustained release of nitrogen oxides by NOS 2 activity did not modify the basal activity of the LTR promoter.

In contrast, the TNF-induced NF-κB activation of the LTR promoter in JJhan-5.1 cells was inhibited 73% by stimulated EMT-6 cells (Table I). The inhibition increased with time, reaching 0, 23, 78, and 90% when JJhan-5.1 cells were cultured with stimulated EMT-6 cells for 1, 2, 4, and 6 h, respectively (mean of triplicates in one experiment, S.D. <5%).

Two NOS inhibitors prevented this inhibition. The best protection against stimulated EMT-6 cells was achieved with aminoguanidine, which inhibited nitrite production more efficiently than l-NAME (Table I). Inhibitors alone had no effect on luciferase expression. A nitric oxide scavenger, carboxy-PTIO, also prevented the decrease in luciferase level (data not shown). Compilation of different experiments revealed a strong correlation between NOS 2 activity and nitrite release, and inhibition of TNF-dependent luciferase induction in JJhan-5.1 target cells (Fig. 1). EMT-6 cells culture supernatants were not inhibitory (data not shown). Taken together, these results indicated that physiologic concentrations of NO (or a related nitrogen oxide) impaired the induction of a LTR-controlled luciferase reporter gene in response to TNF-α. TNF-α binding to JJhan-5.1 cells was not modified after incubation with EMT-6 cells. The receptor affinity (Kd = 1.39 ± 0.28 and 1.35 ± 0.35 μM) and the number of sites per cell (972 ± 111 and 990 ± 110) were similar in JJhan-5.1 cells previously cultured alone or with stimulated EMT-6 cells, respectively (mean ± S.E., n = 2). Thus, the decrease in inducible luciferase expression mediated by stimulated EMT-6 cells did not result from a defect in TNF-α binding to JJhan-5.1 cells.

Like TNF-α, an association of 0.5 μM TPA and 5 μg/ml PHA induces luciferase expression in JJhan-5.1 cells. When these cells were co-cultured with EMT-6 cells stimulated for NO production, subsequent stimulation with TPA and PHA was strongly inhibited, resulting in luciferase expression of 20.9 ± 5.9%, as compared with JJhan-5.1 cells alone. The presence of 2 mM l-NAME partially prevented this inhibition (percent luciferase = 49.8 ± 7.04; mean ± S.E., n = 4; p < 0.01 compared with cells without inhibitor). These results suggested that NO inhibited a common step in the signaling pathways triggered by TNF-α and TPA/PHA, leading to HIV-1 LTR activation.

Since the activity of the viral promoter is dependent on NF-κB activation, whether it is induced by TNF-α or by TPA/PHA, it was postulated that NO might inhibit the LTR-driven luciferase expression via the inhibition of NF-κB activation.

To address this question, electrophoretic mobility shift assays were performed with whole cell extracts from JJhan-5.1 cells. As described previously for the J-Jhan parental clone (29), stimulation with TNF-α induced in JJhan-5.1 cells a DNA binding activity toward a κB oligonucleotide sequence (Fig. 2). Supershift experiments with antibodies against p50, p65(ReA),...
and c-Rel subunits of NF-κB confirmed the predominance of p50/p65(RelA) dimer in the κB binding proteins (not shown). Co-culture of JJhan-5.1 cells for 4 h with stimulated EMT-6 cells followed by the addition of TNF-α strongly inhibited the appearance of the NF-κB binding activity (Fig. 2). Unstimulated EMT-6 cells, which did not express NOS 2 activity, did not modify the response of JJhan-5.1 cells to TNF-α. The NOS 2 inhibitors aminoguanidine and L-NAME prevented the effect of stimulated EMT-6 cells on NF-κB activation. Their efficacy (aminoguanidine > L-NAME) was directly related to their ability to inhibit nitrite synthesis by EMT-6 cells (Fig. 2). It was therefore concluded from these experiments that NO-related species released by EMT-6 cells inhibited NF-κB activation induced by TNF-α and also by TPA/PHA, thereby inhibiting the TNF-induced LTR transactivation.

In an attempt to characterize the NO-derived species involved in inhibition of NF-κB activation, we investigated the effects of two different chemical precursors of nitrogen oxides, GSNO and DETA-NO. DETA-NO releases mostly the radical zNO molecule and exhibits a long half-life (t½ > 2 0ha t3 7° C ) that minimizes the formation of autoxidation products like N₂O₃ (33). GSNO is an S-nitrosothiol, and as such can be considered as a precursor of NO⁺, NO⁻, and even NO⁻⁻-like species, depending on its redox environment. As shown in Fig. 3, GSNO strongly inhibited the activation of NF-κB in JJhan-5.1 cells stimulated with TNF-α. Maximal inhibition occurred between 94 and 187 μM, corresponding to 13 and 32 μM nitrite produced in the medium. On the basis of nitrite production, taken as a rough estimate of total nitrogen oxide output, GSNO was as effective as EMT-6 cells (compare Fig. 2, lane 3, with Fig. 3, lane 4). In contrast, DETA-NO was a very weak inhibitor of NF-κB activation, even at a concentration of 2 mM (Fig. 4).

Since the low pH of the Griess assay accelerated DETA-NO breakdown, measurement of nitrite production was not possible here. Yet the amount of total NO released was estimated from the half-life of DETA-NO, after we had checked that the...
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presence of JJhan-5.1 cells had not modified the value previously reported (not shown). Nitrite and nitrate (NO₃⁻) are the stable end products of NO oxidation. An estimation of the concentration of NO₂⁻ produced within 4 h was therefore calculated (Fig. 4). Even if one assumes a low nitrite:nitrate ratio in NO₂⁻ (e.g. 1:4), 2 mM DETA-NO, which caused less than 50% inhibition of NF-κB activation, would have generated as much as 130 μM nitrite. This is in sharp contrast with GSNO, which displayed a similar inhibitory effect toward NF-κB activation with a 10-fold lower nitrite production (Fig. 3 lane 3). Thus, it seems that the nitrogen oxide species produced from DETA-NO breakdown, and identified as NO itself by others, is only marginally involved in inhibition of NF-κB activation, under our experimental conditions. Accordingly, spermine-NO, which belongs to the same class of “pure” NO donors as DETA-NO but exhibits a shorter half-life (t½ = 39 min) (33), did not inhibit NF-κB activation by more than 50%, up to 500 μM (data not shown).

DISCUSSION

The present study demonstrates that a NOS 2 product inhibits the HIV-1 LTR promoter activity induced by TNF-α or TPA/PHA, in a human T cell line. This inhibition is correlated with a large decrease in NF-κB activation. In contrast, NOS 2 activity did not affect the basal luciferase level. Oxidants like hydrogen peroxide have been reported to down-regulate TNF-α binding (34). Strong oxidants derived from NO, including peroxynitrite, can be produced in living cells. It was therefore important to determine whether TNF-α binding to JJhan-5.1 cells was not modified after co-culture with stimulated EMT-6 cells. Our results indicate that, under our conditions, no reduction in TNF-α receptor affinity or density could be detected. NOS 2 activity also inhibited the induction of luciferase activity by TPA and PHA, a stimulus that did not involve binding to TNF receptors. Both signaling pathways converge on NF-κB activation, supporting the hypothesis that inhibition of NF-κB accounted for the decrease in induction of the LTR luciferase gene.

Previous findings concerning the modulation of NF-κB activation by NO are controversial. Early studies indicated that the chemical NO donors sodium nitroprusside (SNP) and S-nitroso-N-acetylpenicillamine (SNAP) were capable of activating NF-κB in human peripheral blood mononuclear cells (24). This was correlated with an enhancement of protein tyrosine phosphatase activity and with an increase in the kinase activity of p56lck. The same authors demonstrated an enhancement of GTPase activity of the guanine nucleotide-binding protein p21ras after exposure to NO, accompanied by S-nitrosylation of this protein (25, 26, 35). These data led to the hypothesis that NO alone, by inducing the S-nitrosylation of p21ras, activated the G protein which, in turn, perhaps through a protein tyrosine phosphatase/kinase signaling pathway involving p56lck and possibly mitogen-activated protein kinases, ultimately activated NF-κB. Our results did not corroborate this activation pathway. By using the JJhan-5.1 cell line and EMT-6 cells capable of producing sustained levels of nitrogen oxides, we never observed significant activation of the NF-κB transcription factor or induction of the NF-κB-dependent HIV-1 LTR promoter activity by NOS 2 activity. On the contrary, our findings indicate an inhibition of NF-κB activity by nitrogen oxides. Other studies in different models have reported an inhibitory effect of chemical NO donors (SNP or GSNO) on NF-κB activation (19–21). An explanation for these conflicting results, compared with those of Lander’s group (24–26), might be related to the different fluxes of NO used in these experiments. Lander and co-workers (24–26) found that micromolar or submicromolar concentrations of pharmacological sources of NO (NO gas, SNP, and SNAP) were sufficient to activate NF-κB in gel shift assays (24, 26). On the other hand, we used high output murine NOS 2 activity to generate NO under physiological conditions, at a mean rate of 2–4 μM NO/ h. In other reports, inhibitory concentrations of NO donors were frequently 100 times higher than the micromolar amounts shown to activate NF-κB per se (19–21). Therefore, it may be that low amounts of NO would activate NF-κB by themselves in some cell types, whereas high fluxes of NO would be inhibitory.

Our model takes into account the formation of NO-related species such as nitrosothiols, peroxynitrite, and dinitrosyl iron complexes, which would be expected to be produced at the same time as NO in NOS-expressing cells. Although numerous studies have made use of exogenous organo precursors of NO (SNP, SNAP, GSNO, and sydnonimines), only a few experiments have been undertaken to delineate the role of NO produced endogenously by NO activity. NOS inhibitors activated NF-κB in unstimulated endothelial cells (but much so less than TNF-α), suggesting the involvement of NOS 3 activity in NF-κB regulation (21, 23). Inhibition of NOS 2 activity induced by LPS or silica in the mouse macrophage cell line RAW 264.7 resulted in an autocrine enhancement of NF-κB activation in the same cells, consistent with a negative feedback of NO on nos 2 gene transcription (36). Thus our experiments demonstrate for the first time a direct inhibitory effect of NO-related species generated by NOS 2 activity on NF-κB activation, in a paracrine model comprising distinct effector and target cells. In this respect, our results indicate that different NO-related species might greatly differ in their ability to inhibit NF-κB activation. On a concentration basis, the nitrosothiol GSNO is much more efficient than DETA-NO. If the comparison is made on the basis of the estimated rate of nitrogen oxide production, DETA-NO is about 10 times less efficient than GSNO. Since DETA-NO releases mostly the NO radical (33), it thus seems likely that NO itself was not or was only marginally involved in the inhibition of NF-κB activation. The fact that a nitrosothiol like GSNO could mimic the inhibition of NF-κB activation observed with stimulated EMT-6 cells suggests that the relevant inhibitor nitrogen oxide in the co-culture model might also be a nitrosothiol, or at least a nitrosating species. Interestingly, nitrosothiols have been detected in vivo and in cell cultures in vitro (37–39). Moreover, GSNO and SNAP have also been successfully used by other authors (19–21, 23) to inhibit the transcriptional activation of NF-κB-dependent genes. Finally, Peng et al. (27) demonstrated stabilization of IkB-α and transcriptional induction of the IkB-α gene by GSNO, providing a molecular support for the deficient activation of NF-κB in the presence of NO-derived molecules. SNP and SNAP also directly inhibited the DNA binding activity of NF-κB, probably via the nitrosation of a cysteine residue critical for DNA recognition, identified as Cys-62 in the p50 subunit (22). Thus, our experiments showing the inhibition of NF-κB activation by a physiological source of nitrogen oxides, which is stimulated EMT-6 cells, further validate a posteriori those experiments that used S-nitroso derivatives as chemical substitutes for NO synthase activity.

The relevance of the present findings to HIV-1 pathology has to be considered. Our results revealed a significant inhibition of NF-κB-dependent LTR transactivation by a high flux of NO generated continuously for 2–4 h. The main cell targets of HIV viruses are lymphocytes and macrophages. Since NF-κB activity is a major requirement for inducing HIV LTR activity in circulating lymphocytes and in monocytes/macrophages (40, 41), the negative effect of NO on this activation might have important implications in reducing viral replication. The inhibition required a sustained production of nitrogen oxides, pro-
duced by inducible, high output NOS 2 activity. Human lymphocytes have been shown to release only low amounts of NO (10, 42). Induction of NOS 2 in human monocytes/macrophages is controversial (reviewed in Ref. 43). Although there is some evidence that macropages can produce NO at a high level under favorable conditions, NOS 2 activity in HIV-infected monocytes is weak (13). In the human brain, which is dramatically affected by viral toxicity, induction of NOS 2 activity was not observed in microglia, the brain macrophages (44, 45). In contrast, cytokine-activated human astrocytes were reported to express this NOS isoform (44, 45). The activity of fetal astrocytes was high, similar to the murine counterpart (45). NOS 2 activity is expressed in human astrocytes in response to viral membrane components or to inflammatory cytokines such as interleukin-1 and TNF-α, released during HIV-1 infection (14, 44, 46). Astrocytes activated for NOS 2 expression might thus inhibit the NF-κB-dependent HIV-LTR transactivation in other glial cells, in a paracrine manner. The antiviral effect of NOS 2 might limit the reactivation of the HIV-1 provirus, in the same way as it blocks the activation of the latent Epstein-Barr virus genome (10), thereby contributing to a latent HIV-1 infection in glia (14) and participating at the same time in HIV-1 pathology through NO-induced neurotoxicity.

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