Induction of Indolamine 2,3-Dioxygenase in Primary Human Macrophages by Human Immunodeficiency Virus Type 1 Is Strain Dependent

ROSS S. GRANT, HASSAN NAIF, SOPHIE J. THURUTHYIL, NAJLA NASR, TAMANTHA LITTLEJOHN, OSAMU TAKIKAWA, AND VIMAL KAPOOR

School of Physiology and Pharmacology, Faculty of Medicine, University of New South Wales, Sydney 2052, Centre for Virus Research, University of Sydney, Westmead Hospital, Westmead 2145, and Australian Cataract Research Foundation, University of Wollongong, Wollongong 2522, New South Wales, Australia

Received 7 June 1999/Accepted 28 January 2000

A significant percentage of HIV-1 infected individuals develop cognitive/motor abnormalities (16), which are referred to collectively as AIDS-related dementia complex (ADC). In ADC the CNS pathology is characterized by neuronal cell loss, astrogliosis, infiltrating macrophages, and formation of microglial nodules and giant cells (4, 9, 15). However, the underlying cause of neuronal degeneration in ADC is unknown. Productive HIV infection in the CNS is limited to macrophages and microglial cells, with restricted infection in astrocytes and essentially no infection within neuronal cells (16). This suggests that HIV-1 infection of brain macrophages may be central to the loss of neurological function in ADC through an indirect immune system-mediated mechanism (13).

Macrophages activated with HIV-1 or HIV-1 envelope glycoprotein (gp120) contribute to the production of a number of putative neurotoxins including glutamate (5), arachidonic acid metabolites (12, 13), nitric oxide (12), platelet-activating factor (13), tumor necrosis factor alpha (12, 13, 38), and quinolinic acid (30, 36). Elevated levels of quinolinic acid, in particular, have been consistently observed in vivo in the cerebrospinal fluid and brain parenchyma of patients with ADC (1, 19, 36). The severity of neurological symptoms has been correlated with the increase in quinolinic acid levels in the brain in the simian immunodeficiency virus model of ADC (21).

Quinolinic acid is an endogenous agonist (excitotoxin) at the N-methyl-d-aspartate receptor, a subtype of the glutamate receptor in the CNS, and therefore may act as a primary mediator of neuronal dysfunction in ADC (35).

Quinolinic acid is produced de novo by the oxidative catabolism of the essential amino acid tryptophan through the kynurenine pathway (5). The first and rate-limiting enzyme for this pathway is IDO, which can be induced by the proinflammatory cytokine IFN-γ (2, 19, 37). IDO catalyzes the conversion of tryptophan to N-formylkynurenine, which can then be converted nonenzymatically to the first stable product, kynurenine (Fig. 1). An increase in IDO activity has been found in the frontal cortex of patients with ADC but not in HIV-1 infected patients without encephalopathy (35). This suggests that quinolinic acid is synthesized locally in the CNS of these patients and may be one of the factors associated with the unique pathology of ADC.

HIV-1 can be isolated from the CNS of virtually all subjects with AIDS; however, only about 30% of those individuals develop dementia (17). Activated macrophages and microglia are apparently the only cells capable of catabolizing tryptophan to quinolinic acid in the CNS (20, 25). Low levels of quinolinic acid production from HIV-1-infected macrophages have been observed (30, 36); however, neither the mechanism of quinolinic acid synthesis nor the effect of different viral strains on the induction of IDO has been previously investigated. Although the role of strain variability in the development of ADC is unknown, it has been suggested that conservation of key amino acids in the third hypervariable region (V3) of gp120 in brain-derived HIV-1 isolates correlates with their ability to infect brain microglia/macrophages (26, 32). This cellular tropism, which is also influenced by the
expression and utilization of various types of HIV-1 coreceptors (18), may be important in the etiology of ADC.

In this study we investigated whether direct infection of MDM in culture with different HIV-1 strains could induce the first enzyme of the kynurenine pathway, IDO, leading to kynurenine synthesis. The results showed that only selected HIV-1 strains induce IDO and kynurenine pathway metabolism efficiently in MDM and that this did not appear to be related to their level of replication.

**MATERIALS AND METHODS**

Abbreviations used in this paper. ADC, AIDS-related dementia complex; IDO, indolamine,2,3-dioxygenase; MDM, monocyte derived macrophages; IFN-γ, interferon gamma; HIV-1, human immunodeficiency virus type 1; CNS, central nervous system; LA, laboratory adapted; TBS, Tris-buffered saline.

Monocyte isolation. Human peripheral blood monocytes were extracted from 400 ml of whole blood from healthy HIV-1-seronegative volunteers as previously described (23). Briefly, blood mononuclear cells were obtained by differential centrifugation in Ficoll-Hypaque (Pharmacia-AMRAD, Sydney, Australia).

**FIG. 1.** The kynurenine pathway (a) IDO (EC 1.13.11.17). (b) Kynurenine formylase (EC 3.5.1.9). (c) Kynurenine aminotransferase (EC 2.6.1.7). (d) Kynurenine 3-hydroxylase (EC 1.14.13.9). (e) Kynurenamide (EC 3.7.1.3). (f) 3-Hydroxyanthranilic acid oxidase (EC 1.13.11.6). (g) Picolinic acid carboxylase (EC 4.1.1.45). (h) Quinolinic acid phosphoribosyltransferase (EC 2.4.2.19). (i) poly(ADP)polymerase (EC 2.4.2.30). (j) Nicotiamide phosphoribosyltransferase (EC 2.4.2.12). CoA, coenzyme A.
IDO was used as a positive control. This IDO was a recombinant protein expressed in *Escherichia coli* (T. Littlejohn and O. Takikawa, submitted for publication).

**IDO densitometry.** The bands corresponding to IDO (from Western blots) were quantified using a Bio-Rad model GS-700 imaging densitometer and Image Tool software (University of Texas Health Sciences Center, San Antonio, Tex.).

**RESULTS**

**HIV-1 productive infection (p24 antigen).** The infection and replication kinetics of the three HIV-1 strains were compared for up to 8 days postinfection by determining the level of p24 antigen in the culture supernatants. All viral strains produced readily detectable concentrations of p24 antigen by day 8 post infection. The LA macrophage-tropic HIV1-BaL showed a high level of replication by day 8 post infection in MDM from donors 1 and 2. The brain-derived primary isolate HIV1-631 showed an intermediate level of replication by day 8 postinfection in donor 2 MDM (Fig. 2). The LA brain-derived HIV1-JRFL showed a high level of replication in donor 1 MDM, comparable to that observed for HIV1-BaL (data not shown), but an intermediate level of replication in donor 2 MDM (Fig. 2).

**IDO induction and supernatant kynurenine concentration in HIV-1-infected macrophage cultures.** Using Western blot analysis, we assayed for IDO protein in the cell homogenate of macrophages from two different donors infected with different HIV-1 strains. MDM from donor 1 were infected with HIV1-BaL and HIV1-JRFL only, while MDM from donor 2 were infected with HIV1-BaL, HIV1-JRFL, and HIV1-631. Samples were taken on days 1, 2, 5, and 8 postinfection. IFN-γ a potent inducer of IDO in MDM (2, 19), was added to selected cultures as a positive control.

An increase in antibody staining for IDO protein was observed on day 1 postinfection for MDM infected with brain-derived LA HIV1-JRFL or treated with IFN-γ (Fig. 3). On day 2 postinfection, a marked increase in IDO staining intensity was detected in MDM either treated with IFN-γ or infected with the brain-derived LA HIV1-JRFL or the brain-derived primary isolate HIV1-631 (Fig. 3 and 4). MDM infected with the highly replicating macrophage-tropic LA HIV1-BaL showed only a slight increase in detectable IDO above baseline on day 2 postinfection (Fig. 3 and 4). All HIV-1-mediated induction of IDO declined to baseline levels by day 8 postinfection.

To clarify how the induction of IDO by HIV may affect tryptophan metabolism in these cultured MDM, the level of kynurenine, a major metabolite, was measured in the supernatant of these macrophage cell cultures. Supernatant kynurenine levels increased markedly by day 2 post infection in cultures either exposed to IFN-γ or infected with the brain-derived LA strain HIV1-JRFL and the brain-derived primary isolate HIV1-631. However, little or no increase in the kynurenine level was observed in the supernatant of cells infected with LA macrophage tropic HIV1-BaL (Fig. 5). At maximal IDO induction (day 2 post infection, Fig. 4), the level of IDO induction by the different viral strains correlated precisely with the pattern of kynurenine concentration measured in the culture supernatant, HIV1-JRFL > HIV1-631 > HIV1-BaL, where the induction of IDO by HIV1-BaL was only marginally above baseline (Fig. 4).

**Effect of viral replication on IDO induction by exogenous IFN-γ.** IDO induction and kynurenine production in HIV-1 infected MDM decreased to control levels by day 8 postinfec tion, a time when viral replication was most abundant (Fig. 2). To investigate whether viral replication was affecting the mechanism through which cytokines induce IDO, 7-day-old MDM were infected with the three strains of HIV-1. On day 6 postinfection, 600 U of IFN-γ per ml was added to the HIV-1-infected MDM.
infected cultures, and 48 h later samples were taken for Western blot analysis of IDO and measurement of the supernatant kynurenine concentration as described above. IDO was strongly induced by IFN-γ in all HIV-1-infected cultures (data not shown), similar to treatment with IFN-γ alone. The kynurenine concentration in the supernatant of these cultures was also markedly increased (HIV1-BaL plus IFN-γ, 52 ± 2 μM; HIV1-JRFL plus IFN-γ, 55 ± 5 μM), similar to that observed for IFN-γ treatment alone (Fig. 5). These findings indicated that the replication of HIV-1 itself does not affect the induction of IDO by exogenous IFN-γ.

**Effect of anti-IFN-γ antibody on kynurenine metabolism in HIV1-JRFL-infected macrophages.** To investigate the mechanism of IDO induction (leading to kynurenine synthesis), we added a human antibody to IFN-γ (100 U/ml) to cells infected with HIV1-JRFL on day 9 in culture and assayed for both the supernatant kynurenine concentration and IDO protein level after 48 h. Figure 6 shows that the supernatant kynurenine concentration was reduced by greater than 60% in the presence of anti-IFN-γ antibody (Fig. 6A). This was consistent with a complete lack of detectable IDO protein in the cell homogenate (Fig. 6B). The presence of an isotype control for this antibody did not significantly affect kynurenine production in HIV1-JRFL-infected cells (data not shown).

**DISCUSSION**

In this study we have shown for the first time that IDO, the rate-limiting enzyme for oxidative tryptophan catabolism, can be induced by HIV-1 in human MDM. Infection with the brain-derived isolates LA HIV1-JRFL and the primary isolate HIV1-631 but not the macrophage-tropic LA HIV1-BaL induced MDM to produce considerable amounts of IDO and its metabolic product, kynurenine. Addition of an antibody to IFN-γ on the day of infection resulted in undetectable levels of IDO protein in the cell homogenate and a large reduction in kynurenine concentration in the culture supernatant.

HIV infection of the CNS is present in most subjects with AIDS; however, only a subset of these patients develop the neurological symptoms associated with ADC (16). This suggests that not all strains of HIV-1 are able to induce the production of neurotoxins, which have been associated with the neuropathology of ADC (28). Secretion of a number of potential neurotoxins from HIV-1-infected macrophages and microglia (6, 16, 32, 36, 38), including the excitotoxin quinolinic acid (6, 30), has been reported. Quinolinic acid is increased in the CNS of HIV-1-infected patients with dementia but not in patients without neurological symptoms (36).

The development of clinical dementia (32) and the production of quinolinic acid in HIV-1-infected macrophages (6) differ according to the viral strain. Quinolinic acid synthesis is regulated by IDO, the rate-limiting enzyme of the kynurenine pathway (Fig. 1). We have shown, for the first time, that HIV-1 infection results in a significant induction of IDO protein in MDM. Consistent with previous reports regarding increased quinolinic acid production (6), not all strains of HIV-1 were able to induce IDO. The brain derived viruses LA HIV1-JRFL and primary isolate HIV1-631 were able to markedly induce IDO, resulting in a significant increase in kynurenine secretion into the culture supernatant. This may be relevant clinically, since increasing kynurenine pathway metabolism in MDM has been shown consistently to result in elevated levels of quinolinic acid in extracellular fluid (20, 34).

Interestingly, little or no induction of IDO or kynurenine production was observed in cells infected with the highly replicating LA macrophage-tropic strain (HIV1-BaL). From the data above, it appears that the induction of IDO may be related to the viral tropism (i.e., neurotropism); however, a much larger panel of different viral strains must be examined to confirm this hypothesis. These results also suggest that IDO induction in vitro correlates with the viral characteristics associated with in vivo strain adaptation rather than with the level of viral replication (6).
It was noted that both induction of IDO and kynurenine secretion decreased to baseline levels by day 8 postinfection (Fig. 3 and 4), a time when HIV-1 replication was highest (Fig. 2). However, addition of exogenous IFN-γ to HIV-1-infected cultures during this time (i.e., day 7 to 8 postinfec-
tion) resulted in induction of IDO to the same degree as in uninfected IFN-
γ-treated cells. This indicates that productive HIV-1 replication does not affect the induction of IDO if IFN-γ is present in the extracellular fluid. Therefore, the mechanism of IDO induction may involve endogenous production of IFN-γ by MDM in the initial stages of HIV-1 infection, which may then be downregulated by productive viral replication (29).

It is well known that IFN-γ is a potent inducer of IDO and kynurenine pathway metabolism in macrophages (2, 33, 34, 39). T cells and NK cells are considered the primary source of IFN-γ during an immune response (14). Recently, other cell types, including monocyte/macrophages, have been shown to be able to synthesize IFN-γ (3, 14). Therefore, we investigated the possibility that endogenous IFN-γ production may mediate the induction of IDO in these HIV-1-infected MDM. Addition of a human antibody to IFN-γ simultaneously with infection (HIV1-JRFL) resulted in an absence of detectable IDO produc-
tion. This indicates that productive HIV-1 replica-
tion does not affect the induction of IDO if IFN-
γ-treated cells. This indicates that productive HIV-1 replica-
tion is present in

These results suggest that infection of MDM with selected HIV-1 strains (such as HIV1-JRFL and HIV1-631) stimulates the production of endogenous IFN-γ, resulting in immune system activation, IDO induction, and subsequent increased flux through the kynurenine pathway. However, this phenom-
eron was transitory, declining to baseline by day 8 postinfection (Fig. 3 and 4), while HIV-1 replication continued to in-
crease (Fig. 2). In agreement with this result, it has recently been reported that acute HIV-1 infection downregulates IFN-γ expression in activated cells by a DNA methyltransferase-dependent process (29).

In conclusion, we observed that both LA and primary brain-

These results suggest that therapeutic strategies targeted at regulating IFN-γ production may be an alternative approach to managing patients at risk of ADC, in addition to strategies already suggested for dealing with individual downstream ex-
citotoxins such as quinolinic acid (22, 24).

ACKNOWLEDGMENTS

We thank Z. Miklowska for the donation of IFN-γ antibody and Beena Devenapalli, Shan Li, and Mohomed Alali for technical advice and assistance.

This study was supported by grants from the R. L. Cooper Medical Research Foundation. R. S. Grant is supported by a Dora Lush post-

REFERENCES


8. Dreyer, E. B., and S. A. Lipton. 1995. The coat protein gp120 of HIV-1 inhibits astrocyte uptake of excitatory amino acids via macrophage arachi-


15. Ginllan, D., J. Yu, X. Li, D. Tom, J. Li, E. Wendt, S. Lin, R. Schwarz, and C. Noonan. 1996. Study of receptor mediated neurotoxins released by HIV-


tionships to infection with the human immunodeficiency virus. J. Gen. Virol. 70:2661–2672.


Acknowledged by guest http://jvi.asm.org/ on October 22, 2017 from


