Abstract

Intracerebroventricular (i.c.v.) administration of HIV-1 glycoprotein 120 (gp120), the envelope protein used by the virus to gain access into immune cells, induces neurobehavioral alterations in rats. To examine the role of proinflammatory cytokines in mediating these effects, we measured the effects of gp120 on brain proinflammatory cytokine expression and the effects of anti-inflammatory agents, including interleukin-1 receptor antagonist (IL-1ra), pentoxifylline (a TNFα synthesis blocker) and IL-10, on gp120-induced sickness behavior. I.c.v. administration of gp120 induced the expression of IL-1β, but not TNFα, mRNA in the hypothalamus, 3 h after the injection. Pretreatment of rats with IL-1ra, but not with pentoxifylline, significantly attenuated gp120-induced anorexia and loss in body weight, whereas both agents had no effect on gp120-induced reduction in locomotor activity in the open field. Pretreatment with either IL-1ra and pentoxifylline simultaneously, or with IL-10, produced effects that were similar to the effects of IL-1ra alone. Together, these findings indicate that IL-1, but not TNFα, mediates some of the behavioral effects of acute gp120 administration, suggesting that brain IL-1 may be involved in some of the neurobehavioral abnormalities evident in AIDS patients.

Theme: Endocrine and autonomic regulation

Topic: Neural-immune interactions

Keywords: Gp120; Sickness behavior; IL-1; TNFα; IL-10; AIDS

1. Introduction

Infection with the human immunodeficiency virus (HIV) is associated with profound neurobehavioral disturbances [25,49], which have been attributed to the presence of the virus within the brain [24,43]. However, in the central nervous system (CNS), as in the immune system, extensive damage occurs despite low levels of HIV-infected cells [55,60]. This observation suggests that indirect mechanisms, such as the release of a toxic viral product, may be the cause of neurological dysfunction. The most probable factor is shedding of large quantities of glycoprotein120 (gp120), the surface envelope protein used by the virus to gain access into immune cells [14,25,46].

In HIV-infected individuals, gp120 enters the brain during the early stages of disease progression [19]. Once inside the central nervous system, gp120 binds to an activation receptor on glial cells [54], leading to the release of a variety of neuroactive substances, including proinflammatory cytokines [42,66]. HIV gp120 and fragments of gp120 can stimulate interleukin-1 (IL-1), tumor necrosis factor α (TNFα) and IL-6 when added to cultured brain cells in vitro [8,26,36,41,62]. Studies using ex vivo methods demonstrated that intracerebroventricular (i.c.v.) gp120 in rats produces an enhancement in IL-1β expression, which is involved in apoptotic cell death of cortical neurons [5]. Finally, following i.c.v. infusion of native or recombinant gp120 to rats in vivo, IL-1 protein and
bioactivity were increased in several brain areas [50,51,57] and IL-1β and TNFα mRNA levels were increased in the hypothalamus [30].

The role of gp120 in mediating neurobehavioral changes is well documented. Systemic injection of gp120 into neonatal rats was found to retard developmental milestones associated with complex motor behaviors [28]. In adult rats, i.c.v. administration of gp120 produced a marked sickness behavior syndrome, consisting of reduced exploratory behavior, suppressed consumption of food and saccharin solution, and reduced body weight [7]. I.c.v. gp120 also retarded the acquisition of a spatial memory in the Morris water maze [27] and Barnes maze [52], enhanced non-rapid eye movement (characterizing non-REM sleep), decreased REM sleep for several hours postadministration [45,52] and produced memory impairments in hippocampally dependent contextual fear conditioning, but not in hippocampally independent auditory-cue fear conditioning [50].

The aim of the present study was to further examine the mechanisms underlying the outcomes of i.c.v. gp120 by testing its effects on the induction of IL-1β and TNFα genes within the brain, and determining the role of these cytokines in mediating the consequences of gp120 on behavioral parameters. To examine the role of IL-1, we used the specific IL-1 receptor antagonist (IL-1ra), which has been shown to attenuate or block the effects of IL-1β and several other immune challenges on social exploration injected rats. In that experiment no differences were observed in the activity of the two preparations. For both preparations, in experiment 2 we used the specific IL-1 receptor antagonist (IL-1ra), which preparation. In addition, in experiment 2 we used the anti-inflammatories agent IL-1ra [12,20,29,33,47]. Further-

2. Materials and methods

2.1. Subjects

Subjects were male Fischer 344 rats (Harlan–Sprague–Dawley, Jerusalem, Israel), 3–5 months old. Animals were housed 2–3 per cage in an air-conditioned room (23±1°C), with food and water ad libitum for several weeks before the beginning of the experiments. During the behavioral experiments, all manipulations (i.e. injections and initial measurements) were conducted during the first half of the dark phase of a reversed 12 h light–dark cycle (lights off at 07:00).

2.2. HIV-1 glycoprotein120 (gp120) preparation

Full length, glycosylated HIV-1_{1ib} gp120 recombinant viral protein (Advanced Biotechnologies, Maryland, USA or Intracel, WA, USA) was used. The protein had a molecular weight of 120 kD, and was purified under non-denaturing conditions. Purity was 95% (Advanced Biotechnologies) or 90% (Intracel) as estimated by analysis of Coomassie blue stained SDS–PAGE. Experiments 1–4 were conducted with the Advanced Biotechnologies preparation. In addition, in experiment 2 we used the preparation from Intracel in about half of the gp120-injected rats. In that experiment no differences were observed in the activity of the two preparations. For both preparations, the protein was produced using the baculovirus expression system. Protein concentration was 10 000 μg/ml. The final preparation was adjusted to contain 400 μg of gp120 protein per ml, and was kept at −80°C until use. Before each experiment, the preparation was diluted in saline to get the appropriate protein concentrations for i.c.v. injections.

2.3. Stereotaxic surgery and histology

Rats were anesthetized with sodium pentobarbital [55 mg/kg, intraperitoneally (i.p.)] and placed in a stereotaxic apparatus. A burr hole was drilled 1 mm posterior to bregma and 1.5 mm lateral to the midline, and a 26-gauge stainless-steel guide cannula (Plastics-One) was lowered 4 mm below skull surface. The tip of the guide cannula was positioned 1 mm above the lateral ventricle. The guide cannula was secured to the skull with three stainless-steel screws and dental cement, and was closed by a dummy cannula (Plastics-One).

At the end of the experiment, the location of the guide cannulas was verified by injecting a dye (trypan blue) through a cannula. Brains were removed, cut with a scalpel and the spread of the dye within the ventricles was examined. No animals with a misplaced cannula were found.
2.4. Intracerebroventricular microinjections

For several days before the experiment, rats were handled and habituated to the i.c.v. injection procedure, to minimize stress and discomfort during the experiment. During the experiments, freely moving rats were held loosely on the experimenter’s lap, in a manner identical to the habituation procedure. Rats appeared unstressed throughout the injection procedure and usually there was no need for restraining. Solutions were injected into the lateral ventricle through a 33-gauge stainless steel internal cannula (Plastics-One), which was 1 mm longer than the guide cannula. The internal cannula was connected to a microsyringe pump (Harvard apparatus, South Natic, MA, USA) by a PE20 tube. Solutions were administered at a constant rate of 10 µl/min and the injection cannula was removed 1 min following the termination of the injection, to avoid spillage from the guide cannula.

2.5. Determination of brain IL-1β and TNFα gene expression

Total RNA was isolated from the hypothalamus of each rat, using the Rneasy kit (Qiagen). One µg RNA was reverse-transcribed using an oligoT primer and the Superscript II enzyme. A 50-µl PCR reaction (containing 2 mM MgCl₂, 250 ng of each primer, 0.2 mM of dNTP mix and 2.5 units Taq DNA polymerase) was performed using 2 µl of the reverse transcription reaction as template. To ensure that the detected PCR signals were not due to amplification of genomic DNA, control RT-PCR experiments were performed, in which cDNA was synthesized without reverse transcriptase. The primers’ sequences were: β-actin, TTG TAA CCA ACT GGG ACG ATA TGG (+); GAT CTT GAT CAT GAT CCT GGT GCT AGG (−); IL-1β, GTG ATG TTC CCA TTA GAC AGC (+); CTT TCA TCA CAC AGG ACA GG (−); TNFα, TAC TGA ACT TCG GGG TGA TTG GTC C (+); CAG CCT TGT CCC TTG AAG AGA ACC (−). The PCR reaction ran for 40 cycles at 94 °C (45 s), 54 °C (45 s) and 72 °C (90 s), followed by final extension for 7 min at 72 °C. A 20-µl volume (for IL-1β and TNFα) or 5 µl (for β-actin) of the reaction was electrophoresed on a 2% agarose gel and stained with ethidium bromide. Computerized densitometry of the PCR products was calculated as band area (in pixels)×mean density. IL-1β:β-actin and TNFα:β-actin ratios were compared between the various experimental conditions.

2.6. Open field test

The open field apparatus was 95×95-cm wooden box with 60-cm-high walls. The inside of the box was painted black and the floor divided into 25 identical squares. Each rat was placed in the corner of the apparatus and left there for a period of 3 min. An observer blind to the treatment received by the animal recorded instances of line crossing with both hind paws and instances of rearing with both paws up.

2.7. Procedure

2.7.1. Experiment 1: effects of gp120 on brain IL-1β and TNFα gene expression

Three weeks prior to the experiment, rats were implanted with cannulas for i.c.v. injections. On the experiment day, rats were divided into three groups (n=9–11), injected with either saline or gp120 in one of two doses (0.5 or 2 µg protein/rat dissolved in 10 µl of saline), as described before. Gp120 doses were selected based on previous reports, which mapped dose-related effects of gp120 on in vivo production of proinflammatory cytokines [30,57]. Three hours post injection, rats were sacrificed by decapitation. Brains were removed on ice, dissected under sterile conditions and immediately frozen in liquid nitrogen. Determination of brain IL-1β and TNFα gene expression was performed, as described above. The results were analyzed by ANOVA followed by posthoc tests with the Fisher PLSD procedure.

2.7.2. Experiment 2: effects of IL-1ra on gp120-induced sickness behavior

Three weeks prior to the experiment, rats were implanted with cannulas for i.c.v. injections. One week before the experiment, each rat was placed in an individual cage. During the next 6 days, rats received daily handling and habituation to an i.c.v. injection procedure. Baseline food consumption and body weight were measured 2 days before the experiment. Food consumption was measured by giving each rat 250±300 g of food pellets and weighing the remaining food 24 h later. Preliminary experiments showed that food spillage was negligible (<1% of food consumed). Rats were divided into four subgroups (n=22–28/group), matched for mean baseline food consumption and body weight.

On the morning of the behavioral experiment day, food in the cage and body weight were measured. Rats were then immediately injected i.c.v. with either recombinant human IL-1ra (Amgen, Thousand Oaks, CA, USA; 50 µg protein/rat dissolved in 10 µl of saline) or with saline (same volume). Fifteen minutes later, rats were injected i.c.v. with gp120 at a dose of 2 µg protein/rat (dissolved in 20 µl of saline), or with saline (same volume). The high dose of gp120 (2 µg rather than 0.5 µg protein/rat) was chosen for all the behavioral studies in the present report, based on our previous experience with the dose of gp120 required to induce behavioral alterations [7], as well as reports of other investigators [e.g. 50]. Activity in the open field was assessed 4 h after the injection, as described above. Food consumption and body weight were measured 24 h after the injection. The results were analyzed by
ANOVA followed by posthoc tests with the Fisher PLSD procedure.

2.7.3. Experiment 3: effects of pentoxifylline, administered individually or in combination with IL-1ra, on gp120-induced sickness behavior

All aspects of this experiment were similar to Experiment 2, except that three groups of rats were administered with two consecutive injections, the first i.p. and the second i.c.v., with the following respective compounds: Saline (1 ml/kg) + saline (10 μl) (n=30), pentoxifylline (4 mg/kg in a volume of 1 ml/kg) + saline (10 μl) (n=26), and pentoxifylline (4 mg/kg) + IL-1ra (50 μg/rat in a volume of 10 μl saline) (n=18). Fifteen minutes later, about half of the rats within each of these groups were administered i.c.v. with saline (10 μl/rat) and the rest with gp120 (2 μg/10 μl saline/rat). Note that in previous studies we demonstrated that i.p. administration of this dose of pentoxifylline blocked TNFα mRNA induction and protein production in several brain areas, including the hypothalamus, following i.c.v. administration of Mycoplasma fermentans [61,65].

2.7.4. Experiment 4: effects of IL-10 on gp120-induced sickness behavior

All aspects of this experiment were similar to Experiment 2, except that rats (n=5–7/group) were injected i.c.v. with either recombinant rat IL-10 (NIBSC, Potter Bar, UK; 300 ng/rat in a volume of 1 μl) or with saline, just before an i.c.v. injection of either gp120 (2 μg protein/rat dissolved in 20 μl of saline) or saline (same volume).

3. Results

3.1. Experiment 1: effects of gp120 on brain IL-1β and TNFα gene expression

Administration of gp120 produced a significant increase in brain IL-1β gene expression (F_{2,28}=5.44, P<0.05), but not in brain TNFα gene expression (P>0.1) (Fig. 1). Posthoc tests indicated that gp120 significantly increased IL-1β production in the low dose (0.5 μg/rat) (P<0.05). The high dose of gp120 (2 μg/rat) produced a near significant increase in IL-1β production (P<0.06). There was no significant difference between the two doses (P>0.1).

3.2. Experiment 2: effects of IL-1ra on gp120-induced sickness behavior

Administration of gp120 produced a significant reduction in body weight (F_{1,95}=7.36, P<0.01) and a nearly significant interaction for food consumption (F_{1,95}=3.23, P<0.08). Posthoc tests indicated that gp120 produced a significant reduction in the saline-, but not in the IL-1ra-treated group, for both variables.

Locomotor behavior in the open field apparatus was significantly reduced following administration of gp120 (F_{1,86}=10.36 and 4.24, P<0.005 and 0.05, for line crossing and rearing, respectively) (Fig. 2C and D). There was neither a significant effect for IL-1ra nor an interaction between IL-1ra and gp120 administration (P>0.1).

3.3. Experiment 3: effects of pentoxifylline, administered individually or in combination with IL-1ra, on gp120-induced sickness behavior

Administration of gp120 produced a significant reduction in body weight (F_{1,63}=30.56, P<0.000) and in food consumption (F_{1,63}=13.03, P<0.001) (Fig. 3A and B, respectively). There was a significant blocker effect for body weight (F_{1,63}=6.02, P<0.005), but not for food consumption (P>0.1). Posthoc tests indicated that gp120 produced a significant body weight loss and a significant reduction in food consumption in both the saline- and the pentoxifylline-treated groups, but not in the pentoxifylline+IL-1ra-treated group.

Locomotor behavior in the open field apparatus was significantly reduced following administration of gp120 (F_{1,63}=18.03, P<0.000 for line crossing and F_{1,63}=8.00, P<0.006 for rearing) (Fig. 3C and D, respectively). There was a significant blocker effect for rearing (F_{1,63}=4.31, P<0.02), but not for line crossing (P>0.1). Posthoc tests indicated that gp120 produced a significant reduction in line crossing in all experimental groups. Gp120 produced a significant reduction in rearing in both the saline- and the pentoxifylline-treated groups, but not in the pentoxifylline+IL-1ra-treated group.

3.4. Experiment 4: effects of IL-10 on gp120-induced sickness behavior

Administration of gp120 produced a significant reduction in body weight (F_{1,20}=7.02, P<0.01) and in food consumption (F_{1,20}=11.26, P<0.005) (Fig. 4A and B). There was neither a significant effect for IL-10 nor an interaction between IL-10 and gp120 administration (P>0.1). Posthoc tests indicated that gp120 produced a significant reduction in body weight and food consumption for the saline-, but not the IL-10-treated group.

In the open field apparatus, administration of gp120 produced a significant reduction in line crossing (F_{1,20}=6.02, P<0.05), but not in rearing (P>0.1) (Fig. 4C and D). There was neither a significant effect for IL-10 nor an interaction between IL-10 and gp120 administration (P>0.1).
Fig. 1. Effects of HIV-1 glycoprotein 120 (gp120) on brain IL-1β and TNFα gene expression. Rats were injected i.c.v. with either saline (sal) or gp120 (0.5 μg or 2 μg/μl/rat), and were sacrificed 3 h following the i.c.v. injection. Filters were hybridized with IL-1β and TNFα cDNA probes, as well as with a β-actin cDNA probe, to assure that comparable amounts of RNA were loaded. (A) Examples of RT-PCR of IL-1β mRNA isolated from the hypothalamus of individual animals, injected with saline (a), 0.5 μg gp120 (b) or 2 μg gp120 (c). M, marker, *, and **, negative controls, where PCR was performed on RNA samples without RT (*) or without cDNA (**). (B) Mean (±S.E.M.) IL-1β:β-actin ratio. (C) Mean (±S.E.M.) TNFα:β-actin ratio. *, Significantly different from saline-injected controls (P<0.05).

4. Discussion

The results of the present study demonstrate that i.c.v administration of HIV-1 gp120 induced the expression of IL-1β, but not TNFα mRNA in the hypothalamus. These results extend the findings of several previous studies, which examined the effects of gp120 on proinflammatory cytokines induction in vivo and produced somewhat inconsistent results. For example, Opp et al. [45] injected gp120 (100 ng, i.c.v.) in adult rats and sacrificed them 0, 1, 2 and 4 h postinjection. They found a time-dependent increase in IL-1β mRNA expression in the hypothalamus, which was evident only at 2 h post injection. Chronic i.c.v. microinfusion of gp120 via osmotic mini-pumps (100, 500 and 1000 ng for 72 h) was also found to increase IL-1β mRNAs in the hypothalamus of rats [30]. Interestingly, similar to the findings of the present study, the lowest dose of gp120 (100 ng/24 h) already produced a ceiling effect in the increase of IL-1β mRNA levels. The expression in the hypothalamus of several other cytokines was also elevated, including TNFα and the anti-inflammatory cytokines IL-1ra and TGF-β1 [30]. With respect to TNFα, the difference between the results of that study and the findings presented here suggests that this cytokine is induced after chronic, but not acute administration of gp120. In contrast with the results of studies at the mRNA level, no increases in IL-1β protein levels were found in the hypothalamus, at 3 and 6 h after i.c.v. injection of a
Fig. 2. Effects of IL-1ra on gp120-induced alterations in body weight change, food consumption, and behavior in the open field test. Rats were administered i.c.v. with either saline (Sal) or IL-1ra (50 μg/10 μl/rat). Fifteen minutes later, rats within each of these groups were administered i.c.v. with either saline (Sal) or gp120 (2 μg/10 μl/rat). (A) Mean (±S.E.M.) change in body weight and (B) mean (±S.E.M.) consumption of food were measured during the 24-h period following i.c.v. administration. (C) Line crossing and (D) rearing in the open field test were assessed 4 h post i.c.v. injection. *, Significantly different from saline-injected controls (P<0.05). †, Significantly different from saline-injected controls (P<0.005).

much larger dose of gp120 (4 μg), although increases in IL-1β protein in the hippocampus and frontal cortex were found at both time points after the injection [50]. In two other studies [51,57] increased IL-1 bioactivity in the dorsal hippocampus of rats was found immediately following both native and recombinant gp120. Together, these findings indicate that both acute and chronic administration of gp120 induces the production of IL-1 in several brain areas. The inconsistency with respect to the production of IL-1 in the hypothalamus may reflect methodological aspects of sensitivity (in measuring protein levels or bioactivity vs. mRNA production), although the possibility that IL-1 mRNA is induced but not translated cannot be ruled out. The effects of gp120 (particularly acute injection) on TNFα production are less clear, and further experimentation is needed.

The results of the present study indicate that acute i.c.v. administration of gp120 produces substantial behavioral
Fig. 3. Effects of pentoxifylline, administered individually or in combination with IL-1ra, on gp120-induced alterations in sickness behavior. Three groups of rats were administered with two consecutive injections, the first i.p. and the second i.c.v., with the following respective compounds: Saline + saline (Sal + Sal), pentoxifylline + saline (Pent + Sal), and pentoxifylline + IL-1ra (Pent + IL-1ra). Fifteen minutes later, rats within each of these groups were administered i.c.v. with either saline (Sal) or gp120 (2 μg/10 μl/rat). (A) Mean (±S.E.M.) change in body weight and (B) mean (±S.E.M.) consumption of food were measured during the 24-h period following the injections. (C) Line crossing and (D) rearing in the open field test were assessed 4 h post injection. *, Significantly different from saline-injected controls (P<0.05).

alterations, including anorexia and body weight loss, as well as decreased exploratory and locomotor behavior in the open field. These findings replicate and confirm our previous study on the effects of i.c.v gp120 administration [7]. The behavioral effects of gp120 depend on its complex three-dimensional structure, as we have previously shown that heat-inactivation of this compound completely abolishes these effects [7]. The results further demonstrate that pretreatment with IL-1ra attenuated gp-120-induced anorexia and body weight loss, but it did not affect the reduction in open field activity. A similar effect was obtained with IL-10, which suppresses the production of proinflammatory cytokines, in general, and IL-1 in particular [12,20,29,33,47]. These findings suggest that brain IL-1 plays a pivotal role in mediating the effects of i.c.v gp120 on food consumption and body weight. This conclu-
Fig. 4. Effects of interleukin-10 on gp120-induced alterations in body weight change, food consumption, and behavior in the open field test. Rats were administered i.c.v. with either saline (Sal) or IL-10 (300 ng/1 μl/rat). Immediately later, rats within each of these groups were injected i.c.v. with either saline (Sal) or gp120 (2 μg/10 μl/rat). (A) Mean (±S.E.M.) change in body weight and (B) mean (±S.E.M.) consumption of food were measured during the 24-h period following i.c.v. administration. (C) Line crossing and (D) rearing in the open field test were assessed 4 h post i.c.v. injection. *, Significantly different from saline-injected controls (P < 0.05). **, Significantly different from saline-injected controls (P < 0.01). †, Significantly different from saline-injected controls (P < 0.005).

sion is consistent with the present finding that gp120 induces the production of IL-1β in the hypothalamus, which is critically involved in regulation of food consumption and body-weight [56], and with previous reports on the anorexia and body-weight reducing effects of IL-1 in the brain, in general [18,64], and within the hypothalamus, in particular [2,34]. The effects of IL-1β are centrally mediated, as in a previous study we found that a dose of gp120 identical to that used in the present behavioral studies, which was injected peripherally, did not induce any behavioral alterations in rats [7]. Although exogenous administration of IL-1 has been shown to suppress open-field behavior [63], in the present study both IL-1ra and IL-10 had no effect on gp-120-induced open-field suppression. This finding is consistent with a previous report, demonstrating that IL-1ra had no effect on LPS-induced suppression of open-field behavior [2]. Thus, cytokines other than IL-1 may be involved in the effect of immune...
challenges on open-field behavior, and can compensate for the blockade of IL-1’s activity.

In contrast with the effects of IL-1ra, pretreatment with the TNFα synthesis blocker pentoxifylline had no effect on any of the behavioral effects of gp120. Because previous research indicated that TNFα and IL-1β have synergistic effects in producing many of the behavioral and physiological effects of immune challenges [11,59], we also tested the effects of simultaneous blockade of both cytokines on the effects of gp120. A similar approach has been used previously by others [35] and by our group. For example, we demonstrated that suppression of food consumption and body weight induced by i.c.v administration of Myco-
plasma fermentans was abolished by a combined pretreat-
ment with pentoxifylline and IL-1ra, but not by the administration of each of these blockers by itself [65]. Combined administration of pentoxifylline and IL-1ra also prevented the effects of LPS on proceptive behavior and the preference for a sexually active male in female rats [3,4]. However, in the present study the effects of com-
bined pretreatment with IL-1ra and pentoxifylline were quite similar to those produced with IL-1ra by itself. Together, these findings suggest that TNFα is not involved in mediating the behavioral effects of acute gp120 ad-
ministration. Future studies should examine whether this conclusion is also applicable to the role of TNFα in mediating the effects of chronic gp120 administration, which has clearer and more robust effects on brain TNFα expression.

In the present study, pretreatment with i.c.v. IL-10 blocked gp120-induced anorexia and body weight loss, but had no effect on gp120-induced decrease of line crossing in the open field test (the effect of IL-10 on gp120-induced changes in rearing is inconclusive, because in this particular experiment the effect of gp120 on rearing was not statistically significant). To the best of our knowledge, our findings represent the first report that IL-10 can block the behavioral effects of gp120. This finding is consistent with previous reports that i.c.v. IL-10 attenuated LPS-induced body weight loss [9] and reversed LPS-induced reduction in exploratory behavior [44]. Previous studies showed that gp120 induces IL-10 production and secretion both by peripheral blood mononuclear cells [13,37,53] and within the brain [45]. In the periphery, IL-10 was found to downregulate the expression of proinflammatory cytokines and to upregulate the expression of the anti-inflammatory agent IL-1ra [12,20,29,33,47]. Furthermore, i.c.v. injection of recombinant IL-10 inhibited brain IL-1β and TNFα production in response to i.c.v. LPS [23]. Because in the present study the effects of IL-10 were similar to the effects of IL-1ra, it may be suggested that IL-10-induced suppression of IL-1 production underlies the attenuating effects of IL-10 on gp120-induced sickness behavior.

Infection with the HIV virus is associated with various neurobehavioral disturbances [25,49], including anorexia and cachexia, fever, drowsiness and motor disabilities [17]. Cognitive impairments and emotional abnormalities are also common, even in the early stages of the disease [6,32,39,48]. Although the exact mechanisms that underlie these neurobehavioral changes are not clear, it is widely believed that the release of inflammatory agents by HIV-infected macrophages/microglia cells in the brain is primarily responsible for these changes, rather than the direct effects of the virus [21]. Abundant evidence exists that proinflammatory cytokines are elevated in the CNS during all stages of the HIV infection [19,22,40]. The results of the present study support this notion and suggest that gp-120-induced production of brain IL-1, but not TNFα, may be involved in mediating at least some of the neurobehavioral impairments associated with HIV infec-
tion.

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