Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 11246–11250, December 1991
Neurobiology

Human immunodeficiency virus glycoprotein (gp120) infused into rat brain induces interleukin 1 to elevate pituitary–adrenal activity and decrease peripheral cellular immune responses

(AIDS/cytokine/natural killer cell/steroid)

SYAM K. SUNDAR, MARK A. CIERPIAL, LAKSHMI S. KAMARAJU, SHIREE LONG, STEVEN HSIEH, CHRISTINE LORENZ, MARK AARON, JAMES C. RITCHIE, AND JAY M. WEISS*

Department of Psychiatry, Box 3829, Duke University Medical Center, Durham, NC 27710

Communicated by Neal E. Miller, September 19, 1991 (received for review July 12, 1991)

ABSTRACT **Intracerebroventricular (i.c.v.) infusion of glycosylated recombinant gp120, the envelope protein of human immunodeficiency virus, in various doses (100 ng to 4 μg) resulted in detection of interleukin 1 (IL-1) activity in a high percentage (61%; 33 of 54) of rat brains, whereas IL-1 was very rarely detected in brains of animals infused with several control substances (4%; 1 of 28). To detect IL-1, clarified glial lysate of diencephalon plus brainstem was subjected to gel exclusion chromatography and fractions were assessed for thymocyte stimulation. IL-1 was seen 2, 6, and 24 hr postinfusion. i.c.v. gp120 also produced known effects of IL-1 in brain, elevating steroid concentration in plasma and decreasing cellular immune responses [natural killer (NK) cell activity and mitogenic response to Con A] of blood and splenic lymphocytes. When gp120 was infused together with α-melanocyte-stimulating hormone (20 ng), which blocks many biological actions of IL-1, gp120 no longer elevated steroids or decreased NK cell activity. After intravenous gp120, IL-1 was not found in brain or plasma, indicating that stimulation of IL-1 in brain by i.c.v. gp120 was not due to gp120 affecting infiltrating cells from blood or to elevated circulating IL-1. That induction of IL-1 in brain might have resulted from lipopolysaccharide (LPS) in the gp120 solution was ruled out by studies showing that (*i*) heating of the infusion solution, which does not affect the capacity of LPS to induce IL-1, eliminated the ability of gp120 infusion to induce brain IL-1, and (*ii*) gp120 induced IL-1 in brains of LPS-resistant C3H/HeJ mice. Injection of gp120 directly into the hippocampus stimulated IL-1 more readily than i.c.v. infusion. Thymocyte stimulation produced by active fractions of gp120-infused brains was blocked by monoclonal antibody to IL-1 receptors. These findings indicate that elevation of IL-1 in brain can result from infection with human immunodeficiency virus and may be responsible for certain abnormalities (e.g., elevated activity of pituitary–adrenal axis) seen in AIDS patients.**

Human immunodeficiency virus (HIV) enters the brain soon after infection with this virus (1). As a result, the various protein constituents of the virus will enter the brain, including gp120, the envelope protein of HIV that binds to the CD4 cell-surface antigen. HIV and gp120 have been shown to induce synthesis of interleukin 1 (IL-1) in peripheral blood monocytes (2, 3). The results described here show that introduction of gp120 into the rat brain rapidly induces IL-1 within the brain. IL-1, a cytokine originally isolated from macrophages, is now known to be released by cells in the brain (4, 5) and is possibly found in neurons (6); IL-1 acting in brain has a variety of biological effects, including induction of fever (7), slow wave sleep (8, 9), and potent stimulation of the pituitary–adrenal (P–A) axis via release of corticotropin-releasing factor (CRF) (10–13). Also, introduction into brain of femtomole quantities of IL-1 or induction of endogenous IL-1 in brain rapidly suppresses peripheral cellular immune responses (14) via CRF-mediated activation of both the P–A axis and the sympathetic nervous system (15, 16). The results presented here also show that, consistent with the brain actions of IL-1 described above, introduction of gp120 into the brain elevates plasma steroids and suppresses cellular immune responses.

METHODS AND MATERIALS

Subjects. Male Sprague–Dawley rats weighing 200–300 g were housed two per cage in enclosed, microisolator cages within laminar-flow racks originally designed by Riley *et al.* (17). Animals were maintained on a 12-hr light (7:00 a.m. to 7:00 p.m.)/12-hr dark (7:00 p.m. to 7:00 a.m.) cycle.

Infusion Procedure. Animals were infused through a cannula placed stereotaxically into the brain [for details of cannula construction, surgery, and infusion, see Weiss *et al.* (18)]. In some experiments, the cannula was cemented to the skull and the infusion was administered to an awake animal 4–6 days later; in other experiments, anesthetized animals were infused immediately after the cannula was appropriately positioned into the brain. The substance infused was introduced slowly over 10 min (for vol of 20 μl or less) or 15 min (40 μl). Except in experiment 1, in which effects were studied at different times after infusion, animals were sacrificed 2.5 hr after the infusion was completed.

Detection of IL-1 in Brain. Under halothane anesthesia, animals were perfused transcardially with 0.9% saline. IL-1 was detected in brain by the procedure of Fontana *et al.* (19). The brain was removed aseptically, meninges were discarded, and brainstem plus diencephalon (a region found to show IL-1 activity in earlier studies) was retained for analysis. Brain tissue was passed sequentially through sterile nylon wool mesh (210 and 132 μm) to obtain dissociated cells rich in astroglia but also containing phagocytic cells including microglia (see refs. 20 and 21). Cells were sonicated (25×10^6 cells per ml) in ice-cold RPMI medium containing protease inhibitors and centrifuged at 100,000 × *g* for 60 min. To remove small molecular mass substances (<10 kDa) and to concentrate (20-fold), the supernatant was subjected to ultrafiltration using Amicon membranes.

The supernatant was then subjected to Sephadex G-50 chromatography essentially as described by Cannon and Dinarello (22). Sephadex G-50 (fine) was packed into glass

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: HIV, human immunodeficiency virus; IL-1, interleukin 1; P–A, pituitary–adrenal; PHA, phytohemagglutinin; NK, natural killer; α-MSH, α-melanocyte-stimulating hormone; LPS, lipopolysaccharide; mAb, monoclonal antibody.

*To whom reprint requests should be addressed.

columns (1 × 40 cm) at a flow rate of 1 ml/min, and the gel was equilibrated with eluting RPMI 1640 medium containing 25 mM Hepes buffer and 5×10^{-5} M 2-mercaptoethanol. Column preparation and fractionation were carried out under sterile conditions in a laminar flow hood. Three hundred microliters of supernatant was loaded onto a column and fractions (one fraction per min) were collected at a flow rate of 1 ml/min. Columns were calibrated using a calibration kit that consisted of substances of known molecular weights (Sigma). Fractions were tested, in triplicate, for IL-1 bioactivity in the thymocyte comitogenic assay as described below.

Thymus was collected from 4- to 6-week-old, endotoxin-resistant C3H/HeJ mice (The Jackson Laboratory), and thymocytes were prepared by mincing the tissues between two sterile glass slides. The thymocytes were resuspended at a concentration of 10×10^6 cells per ml in RPMI 1640 medium containing 5% heat-inactivated fetal bovine serum and phytohemagglutinin (PHA) (1 μg/ml). To each well of 96-well U-bottomed microtiter plates (Flow Laboratories) 100 μl of PHA-treated thymocytes and 100 μl of each fraction were added. After incubation for 68 hr at 37°C, 1 μCi of radioactive thymidine (1 Ci = 37 GBq) (NEN) was added to each well, and plates were incubated for an additional 4 hr. The cultures were then harvested onto glass fiber filters and incorporation of [^{3}H]thymidine was determined in a scintillation counter.

Immunological and Plasma Steroid Assays. For certain experiments, a blood sample was drawn by cardiac puncture before perfusion and/or the spleen was removed after clamping the splenic artery. Mononuclear cells were separated on Ficoll/Hypaque density gradients. Viable lymphocytes were determined by trypan blue dye exclusion. Natural killer (NK) cell activity was determined by the method of Reynolds *et al.* (23). To determine lymphocyte response to a mitogen, 0.1-ml aliquots of lymphocyte suspension [2×10^6 cells per ml in RPMI 1640 medium containing 10% (vol/vol) heat-inactivated fetal calf serum] were mixed with Con A at 10 μg/ml. After 3 days, 1 μCi of [^{3}H]thymidine was added for 4 hr, cells were then harvested, and the amount of incorporated [^{3}H]thymidine was determined. Plasma corticosterone was measured as described by Naylor *et al.* (24).

Statistical Analysis. The criterion for positive IL-1 activity was that stimulation of thymocytes (counts of ^{3}H radioactivity) in one or more of fractions 11–16 had to be at least twice the highest counts found in any of the first six fractions (which eluted prior to void volume) and the PHA-alone control. (The highest value of the first six fractions and the PHA-alone control is therefore referred to as the baseline.) Tests for significant differences between groups were conducted by *t* tests or by Fisher's exact probability.

RESULTS

The initial study determined the effects of infusing (via indwelling lateral ventricle cannula) different doses of recombinant glycosated gp120 (III B; American Bio-Technologies, Cambridge, MA) and sacrificing animals at different intervals after infusion. The gp120 used was secreted by an sf9 insect cell line and was then highly purified by antibody affinity chromatography before placement in phosphate-buffered saline vehicle at a concentration of 100 ng/μl; this concentration was used for all infusions. Three experiments ($n = 12$ in each) were conducted. In the first, animals were infused with 4 μg of gp120 (or 0.9% saline as control) and were sacrificed 2, 6, or 24 hr after infusion (at each time point, three animals were infused with gp120 and one was infused with saline). In each of two subsequent experiments, animals were infused with 100 ng ($n = 4$) or 1.0 μg ($n = 4$) of gp120, or with 0.9% saline ($n = 4$) and were sacrificed 2 hr postinfusion (second experiment) or 6 hr postinfusion (third experiment). Infusions were carried out so that animals were sacrificed between 8:00 a.m. and 10:00 a.m.

Fig. 1 shows effects on plasma corticosterone concentration and NK cell activity. Infusion of gp120 elevated plasma corticosterone, with the most consistent effect being seen at the earliest time point (2 hr) after infusion. The duration of effects decreased as the dose of gp120 given was reduced. NK cell activity was decreased in lymphocytes taken from both blood and spleen 2 hr postinfusion and was decreased in blood lymphocytes at 6 hr. When the mitogenic response to Con A was also measured (6 hr postinfusion experiment), this was significantly reduced in blood lymphocytes of animals infused with 1.0 μg of gp120 (data not shown).

Fig. 2 (*Top*) demonstrates detection of IL-1 activity in the thymocyte assay after fractionation. Fig. 2 (*Bottom*) shows results from analysis of six animals whose brain tissue was fractionated on the same day and assayed together using the same pooled thymocytes; IL-1 activity was clearly evident in animals infused with 1 μg of gp120. In this initial study, a total of 28 brains were analyzed (8 saline infused and 20 gp120 infused); IL-1 was detected in 11 gp120 infused vs. 1 saline infused ($P < 0.05$ by Fisher's exact test). With respect to IL-1 detection, neither a clear time–response nor dose–response was seen after intraventricular gp120: for animals sacrificed 2, 6, and 24 hr after infusion, number of animals that were IL-1 positive/number of animals assayed was 5/10, 4/7, and 2/3, respectively; for the doses of 100, 1, and 4 μg, the numbers were 3/6, 3/5, and 5/9, respectively.

Blockade by α-Melanocyte-Stimulating Hormone (α-MSH). α-MSH blocks many of the biological actions of IL-1, including elevation of plasma steroid concentration and suppression of cellular immune responses produced by IL-1 in brain (25–28). To determine whether elevated steroids and suppressed cellular immune responses after introduction of gp120 into brain resulted from elevated IL-1, α-MSH (20 ng) was infused together with gp120. Since α-MSH does not interfere with synthesis of IL-1 (25), the brains of all animals

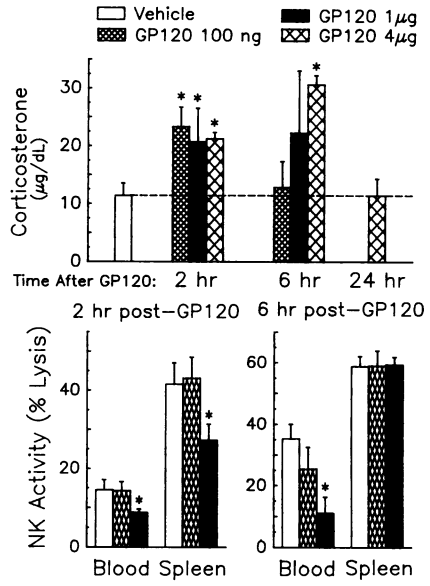

FIG. 1. (*Upper*) Mean (+ SE) plasma corticosterone concentration in animals infused with various doses of gp120 and measured 2, 6, and 24 hr after infusion. Results are combined from three experiments; $n = 9$ (vehicle), $n = 9$ (2 hr), $n = 9$ (6 hr), $n = 3$ (24 hr). (*Lower*) NK activity of animals sacrificed 2 hr ($n = 4$ per group) and 6 hr ($n = 4$ per group) after gp120 infusion. Results with an effector/target cell ratio of 100:1 are shown; other effector/target cell ratios (not shown) yielded similar results. [Note: 2 and 6 hr post-gp120 each represents a separate experiment in which all data shown were obtained in the same assay; since the 4-μg dose was used in a separate (third) experiment, these data do not readily combine with the results shown above and therefore the NK response to 4 μg is not shown.] *, Significantly different from vehicle infused (at least $P < 0.05$).

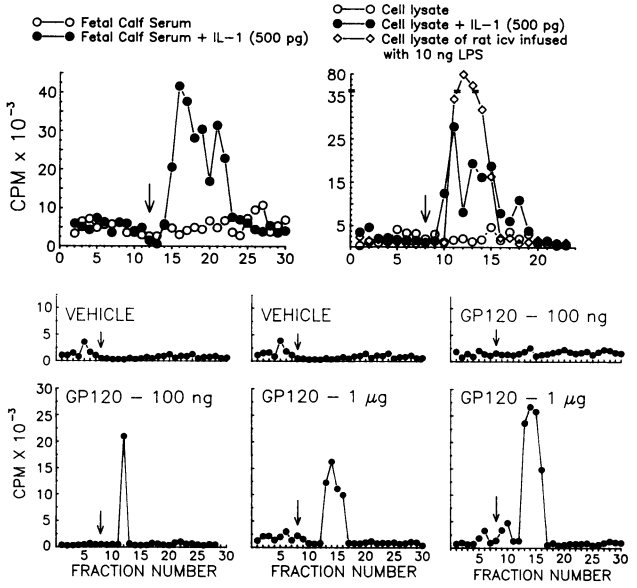

FIG. 2. (*Top*) Verification of the thymocyte stimulation assay for detection of IL-1. (*Left*) Fetal calf serum (0.5 ml) with and without IL-1 was fractionated on a Sephadex 50 column and fractions were then tested for the ability to stimulate thymocytes. (*Right*) Results from fractionation of brain tissue are shown. A marked response occurs from exogenous or endogenous IL-1 (stimulated by LPS), the peak responses evident in the fourth to sixth fraction after the void volume (arrow). The Sephadex column used for fractionation of brain tissue was 5 cm shorter than that used to fractionate fetal calf serum, which accounts for the earlier appearance of void volume (determined by exclusion of dextran blue dye) in brain fractions. (*Middle* and *Bottom*) Response of brain extracts from six animals infused (into the lateral ventricle) with vehicle (n = 2), 100 ng of gp120 (n = 2), and 1.0 μg of gp120 (n = 2), and then sacrificed 2 hr later. Brain tissue of the six animals was fractionated on the same day and thymocyte stimulation using the same pooled thymocytes was initiated on that day. Thymocyte stimulation corresponding to IL-1 activity is evident in both animals infused with 1.0 μg; also, a less pronounced response was seen in one animal infused with 100 ng.

in this study that were infused with gp120 were assayed for IL-1. The findings are shown in Fig. 3. When animals were infused with gp120 (2 or 4 μg) via indwelling ventricular cannula and sacrificed 2.5 hr after infusion, 12 of 20 animals were positive for IL-1, a similar percentage as found in the initial study. Plasma steroid concentration was markedly elevated and NK cell activity of blood lymphocytes was reduced in animals infused with gp120 in which IL-1 activity was detected in brain. Infusion of α-MSH together with gp120 blocked both the steroid elevation and the reduced NK activity of animals whose brains were positive for IL-1. Infusion of α-MSH and 0.9% saline was without effect.

Intravenous Infusion with gp120. To determine whether gp120, even though infused into the lateral ventricle, might have influenced IL-1 in brain through action in the periphery, gp120 was infused intravenously (i.v. via tail vein). gp120 (4 μg; infused over 15 min) was given to five halothane-anesthetized animals and the subjects were sacrificed 2.5 hr later. IL-1 was not detected in any of the five brains. Blood (0.3-ml aliquot) was also fractionated and was negative for IL-1 activity in all cases except one in which a single fraction showed a positive response. In comparison to i.v. saline-infused animals (n = 3), plasma steroids were not elevated (gp120-infused, mean ± SEM = 14.1 ± 2.4 μg/dl; saline-infused, mean ± SEM = 18.7 ± 1.1 μg/dl). These results indicate that induction of IL-1 in brain by infusion of gp120 was not due to the effects of gp120 on peripheral or blood cells. i.v. gp120 did cause some effects, however. In all subjects, a lysate of spleen cells showed IL-1 activity,

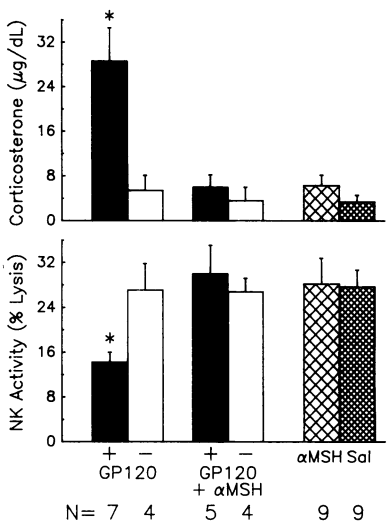

FIG. 3. (*Upper*) Plasma corticosterone concentration and (*Lower*) Blood NK cell activity (mean + SE) for animals infused with gp120, gp120 + α-MSH, α-MSH alone, or saline (0.9%) when sacrificed 2.5 hr after infusion. For gp120-infused animals, subjects are divided into those in which IL-1 was detected in brain and those in which it was not detected; number of subjects in the various groups is indicated below the bars. Amount of gp120 infused was 2 μg (first replication) and 4 μg (second and third replications); 20 ng of α-MSH was given in all cases. Effector/target cell ratio shown for NK cell activity is 100:1. *, Significantly different from each of the other groups (at least $P < 0.05$).

although the response was uniformly weak (i.e., the counts in one or two of the appropriate fractions was only slightly more than twice the baseline). Also, blood NK cell activity was reduced [e.g., percentage lysis (effector/target cell ratio, 200:1) for gp120 infused was 28.7 ± 4.4 vs. saline-infused was 53.2 ± 2.5; t = 3.6; $P < 0.01$].

Potential Confounding Issues: Indwelling Cannulation and Lipopolysaccharide (LPS) Contamination of gp120 Solution. Two potential confounding issues were addressed in overlapping experiments. First, it has been hypothesized that contact with blood induces expression of the CD4 antigen in brain microglia (29). Increased expression of the CD4 antigen in microglia was reported 3 days after disruption of the blood–brain barrier, with peak antigen expression seen after 5 days. Consequently, implantation of an indwelling cannula, which would expose adjacent cells to blood, may have increased CD4 expression in these cells 4–6 days later when gp120 was infused, thereby enabling gp120 to induce IL-1. To determine whether a surgical implantation 4–6 days prior to infusion enabled gp120 to induce IL-1, animals were infused with gp120 (i.c.v., 4 μg) while they were in the stereotaxic instrument immediately after the cannula was positioned. The animals were then sacrificed 2.5 hr later. Of six animals tested, IL-1 activity was detected in the brains of five. Thus, inadvertent exposure of brain cells to blood several days before gp120 infusion to induce CD4 antigen expression does not account for induction of IL-1 in brain by gp120.

Contamination by LPS. Second, since LPS is a potent stimulator of IL-1, it was necessary to determine whether contaminating LPS in gp120 solution was responsible for IL-1 activity in brain. Wahl *et al.* (2) reported that gp120 stimulated release of IL-1 from human blood monocytes *in vitro*; this finding was an important stimulus for the present study. However, Molina *et al.* (30) reported that this effect appeared to depend on the presence of LPS in the gp120 solution. The gp120 used in studies described here is secreted from an sf9 insect cell line so that its generation involves neither lysis of cells nor serum, which is a potential source of LPS; also, after

purification of the gp120 under sterile conditions by immunoaffinity chromatography, no LPS was detectable by limulus assay. Nevertheless, additional experiments were conducted to examine this issue.

First, the ability of heat-treated (Δ) gp120 solution to induce IL-1 from macrophages *in vitro* was assessed. Initial studies indicated that heating (80°C–90°C for 30 min) rendered gp120 unable to induce IL-1 in the brain. However, heating has been reported not to affect the ability of LPS to stimulate IL-1 (31); therefore, the possible presence of LPS could be assessed by determining whether Δgp120 solution would stimulate IL-1. Macrophages were collected by peritoneal lavage of thioglycolate-injected rats and incubated *in vitro* with various concentrations of LPS (1.0, 0.1, 0.001, and 0.0001 μg/ml), the same concentrations of ΔLPS, and Δgp120 solution (40 μl, the largest amount used in these studies, and 80 μl, twice the largest amount). After 48 hr, the presence of IL-1 in macrophage supernatant was tested for IL-1 in the thymocyte stimulation assay. LPS potently activated IL-1 in macrophages *in vitro* in a dose-dependent manner. Equivalent thymocyte stimulation was produced by ΔLPS, thus confirming that heating of LPS did not diminish its ability to activate IL-1. Of most interest, the Δgp120 solution was not devoid of activity in this assay, although the activity produced was quite low, being less than that produced by the lowest concentration of LPS used in this study. A regression analysis using the thymocyte stimulation produced by different concentrations of LPS indicated that the Δgp120 solution produced stimulation equivalent to 1 pg of LPS per μl. (It needs to be clearly noted, however, that the ability of Δgp120 solution to induce IL-1 in macrophages *in vitro* does not mean that LPS was responsible for this induction; Δgp120 peptide could be capable of stimulating IL-1 in macrophages.)

Based on the foregoing, animals were infused with Δgp120; this was done via a chronic indwelling cannula as well as acutely. In addition, low doses of LPS equivalent to (or twice) the LPS concentration that the previous *in vitro* study indicated would account for the thymocyte stimulation of Δgp120 solution were also infused. No IL-1 was detected in 11 animals after infusion of Δgp120 acutely ($n = 6$) or via chronic cannula ($n = 5$); this compares with IL-1 detected in a total of 33 of 54 brains from animals infused with gp120 [difference in percentage of animals that were IL-1 positive when infused with gp120 vs. Δgp120 is statistically significant ($P < 0.001$ by Fisher's exact test)]. Also, no IL-1 activity was detected in brains of any of six animals infused with low doses of LPS [4 with 40 pg (i.e., equivalent to 1 pg/μl in a 40-μl infusion) and 2 with 80 pg].

IL-1 Stimulation by gp120 in Brains of LPS-Resistant C3H/HeJ Mice. As a second test of whether induction of IL-1 in brain by gp120 might be caused by LPS, gp120 was infused into endotoxin-resistant C3H/HeJ mice. C3H/HeJ mice are resistant to effects of bacterial LPS; therefore, any residual LPS in the gp120 solution would be less likely to induce IL-1 in these animals. Immediately after anesthesia with pentobarbital (25 mg/kg), C3H/HeJ mice were placed into the stereotaxic instrument and 0.5 μg (5 μl) of gp120 was infused directly into the hippocampal region. Two and one-half hours later, animals were perfused and the brain tissue surrounding the infusion site (≈60 mg) was analyzed for IL-1 activity. IL-1 activity was detected in five of six animals infused with gp120. IL-1 activity was not detected in any of six animals infused with Δgp120. Also, no activity was detected in two animals infused with a large amount (500 pg) of LPS, confirming that this particular strain is resistant to LPS.

In summary, endotoxin does not appear to be responsible for the induction of IL-1 by gp120 in these studies. Neither heat-treated gp120 solution nor trace amounts of LPS was found to induce IL-1 in brain *in vivo*, and the gp120 also induced IL-1 in the brain of LPS-resistant mice.

Direct Injection of gp120 into Brain Tissue. gp120 was injected directly (by acute procedure) into the dorsal hippocampus of five rats [coordinates: (flat skull) posterior, 5.0 mm (from bregma); lateral, 2.5 mm; depth (from top of skull), 4.0 mm]. The hippocampus was chosen based on studies showing a high concentration of IL-1 receptors in this brain region (32–34). For this procedure, a smaller amount of gp120 [0.5 μg (5.0 μl) infused over 10 min] was given than was typically used for i.c.v. infusion. Two animals were similarly infused with an equal amount of Δgp120. Two and one-half hours after completion of the infusion, each animal was perfused, and a unilateral (side of infusion) segment of dorsal hippocampus (50–60 mg) was analyzed for IL-1 activity. IL-1 activity was detected in all five animals infused with gp120; no activity was detected in the hippocampal region of the two animals injected with Δgp120.

Blocking of gp120-Induced IL-1 Activity by Antibody to IL-1 Receptor. To further determine that the activity observed in the thymocyte stimulation assay was due to IL-1, experiments examined whether a monoclonal antibody (mAb) to mouse IL-1 receptor would block the thymocyte stimulation produced by cell lysate from gp120-infused brain. mAb specific to types 1 and 2 mouse IL-1 receptors was used (Genzyme). At a concentration of 10 μg/ml, mAb blocked the thymocyte stimulation produced *in vitro* by human recombinant IL-1β {[^{3}H]thymidine incorporation expressed as percentage of baseline radioactivity (i.e., PHA alone); 625 pg of IL-1 = 2173% vs. mAb + 625 pg of IL-1 = 188%; 62.5 pg of IL-1 = 493% vs. mAb + 62.5 pg of IL-1 = 99%}. The addition of this antibody did not interfere with the assay; this was shown by testing responsivity of thymocytes to IL-2. Maximal thymocyte stimulation was achieved by 20 ng of IL-2 (95×10^3% of PHA alone), and this response was undiminished in the presence of 10 μg of mAb per ml (118×10^3% of PHA alone). Six animals were then infused with gp120: two rats with 4 μg i.c.v. (acute cannulation) and two rats and two C3H/HeJ LPS-resistant mice with 0.5 μg injected into the hippocampus. Brain tissue (brainstem plus diencephalon for i.c.v. infused and ≈60 mg of hippocampal region tissue for injections) was removed from perfused animals 2.5 hr later, after which brain cell lysate was fractionated and tested for IL-1 activity as described. The results shown in Fig. 4 revealed that IL-1 activity, present in five of the six animals, was blocked by mAb to IL-1 receptor, thereby indicating that thymocyte stimulation in gp120-infused brain was due to IL-1.

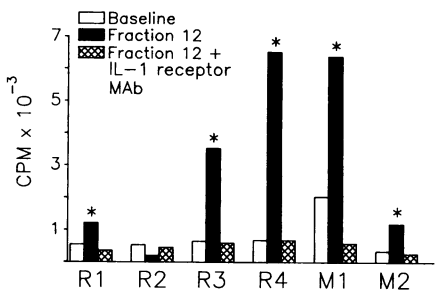

FIG. 4. Thymocyte stimulation (cpm of [^{3}H]thymidine) of the Sephadex column fraction that showed the highest activity in this experiment (fraction 12) and activity of the same fraction incubated with antibody to IL-1 receptor (mAb). Brain tissue was removed for fractionation 2.5 hr after infusion of 4 μg of gp120 into the lateral ventricle [rat (R) 1 and 2], injection of 0.5 μg of gp120 into rat hippocampus (R3 and R4), or injection of 0.5 μg of gp120 into hippocampus of LPS-resistant C3H/HeJ mice (M1 and M2). The baseline for each animal is the highest value observed in the first six fractions of PHA alone. *, Response reaches criterion for IL-1 activity; i.e., twice baseline value.

DISCUSSION

IL-1 has been detected in the cerebrospinal fluid of HIV-infected individuals (35); its presence was noted in 58% of the patients tested. Elevated activity of the P–A axis, marked by increased plasma concentration of both corticotropin and cortisol, has been reported in early-stage HIV infection (stages CDC II and III) (36). The present study indicates that gp120 induces IL-1 in brain, which raises the possibility that elevated IL-1 in brain and increased activity of the P–A axis in HIV-positive individuals are, at least in part, a consequence of gp120 actions in the brain.

The initial studies described here did not show a dose–response relationship between the amount of gp120 that was infused and whether IL-1 was detected in the brain or not. In these experiments, a similar percentage of animals were positive for IL-1 in brain after infusion with either 100 ng, 1.0 μg, or 4.0 μg of gp120 (≈60% of animals at each dose). Interestingly, roughly the same percentage of HIV-infected patients (almost all with fully developed AIDS) were positive for IL-1 in cerebrospinal fluid (35). These results suggest that individual differences in some variable as yet unknown are more critical in determining whether a subject will elaborate IL-1 in brain in response to gp120 than is the amount of gp120 in the brain. On the other hand, subsequent experiments reported in this paper suggest another possibility for the present studies. When gp120 was infused acutely (rather than via a previously implanted cannula), 86% of the rats were IL-1 positive, and when gp120 was injected directly into the hippocampus, 100% of animals were positive despite the infusion of a lower dose (0.5 μg) than those used in most of the studies with i.c.v. infusion. This suggests the following: when gp120 is injected into the ventricular system, it stimulates IL-1 poorly, whereas gp120 is much more effective if it reaches tissue outside the ventricle. Thus, absence of a dose–response relationship in the initial studies may have been due to the vagaries of cannula placement; the ability of gp120 to stimulate IL-1 may have depended on whether the gp120 was delivered totally within the ventricular system or gained access to adjacent tissue by leakage around the cannula shaft or positioning of the cannula tip so that it was partially outside the ventricle. gp120 delivered directly into brain tissue might induce IL-1 more effectively simply because the concentration of gp120 in contact with brain cells is higher than occurs when gp120 is diluted by infusion in the ventricular system; these questions remain to be examined.

Induction of IL-1 in the brain by gp120 could be involved in the pathogenesis of AIDS in a number of ways. First, IL-1 in the brain suppresses various cellular immune responses, which might well render infected individuals susceptible to opportunistic infections. Activation of T-helper cells in response to these infections will activate latent HIV infection, which will, in turn, hasten depletion of $CD4^+$ cells by lysis. Second, HIV infection causes pathological changes in the brain, resulting in psychological and behavioral disturbances (e.g., AIDS dementia). IL-1 acting in brain markedly stimulates the P–A axis, and elevated plasma steroids appear to be neurotoxic, especially when acting in concert with other neurotoxic agents (37). Interestingly, a prime target for this neurotoxicity is the hippocampus, an important brain region involved in memory and cognition, which has a large concentration of receptors that bind glucocorticoids (38–40). Whether such brain changes are significant in HIV-infected individuals remains to be seen since the most common neuropathological features seen in HIV infections are abnormalities of white matter and subcortical structures without loss of neurons; however, these abnormalities do not appear to explain the wide range of psychopathology that has been observed in HIV-infected individuals (e.g., see refs. 1 and 41).

This work was supported by U.S. Public Health Service Grant MH45675 and by funds from the Behavioral Medicine Research Center of Duke University.

1. Perry, S. W. (1990) *Am. J. Psychiatry* **147**, 696–710.
2. Wahl, L. M., Corcoran, M. L., Pyle, S. W., Arthur, L. O., Harel-Bellan, A. & Farrar, W. L. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 621–625.
3. Merrill, J. E., Koyanagi, Y. & Chen, I. S. Y. (1989) *J. Virol.* **63**, 4404–4408.
4. Fontana, A. & Grob, P. (1984) *Lymphocyte Res.* **3**, 11–16.
5. Giulian, D. & Lachman, L. B. (1985) *Science* **228**, 497–499.
6. Breder, C. D., Dinarello, C. A. & Saper, C. B. (1988) *Science* **240**, 321–324.
7. Dinarello, C. A. (1984) *Rev. Infect. Dis.* **6**, 51–95.
8. Krueger, J. M., Walter, J., Dinarello, C. A., Wolff, S. M. & Chedid, L. (1984) *Am. J. Physiol.* **246**, R994–R999.
9. Tobler, I., Borbely, A. A., Schwyzer, M. & Fontana, A. (1984) *Eur. J. Pharmacol.* **104**, 191–192.
10. Bernton, E. W., Beach, J. E., Holaday, J. W., Smallridge, R. C. & Fein, H. G. (1987) *Science* **238**, 519–521.
11. Berkenbosch, F., Oers, J. V., Rey, A. D., Tilders, F. & Besedovsky, H. (1987) *Science* **233**, 652–654.
12. Saplosky, R., Rivier, C., Yamamoto, G., Plotsky, P. & Vale, W. (1987) *Science* **238**, 522–524.
13. Besedovksy, H., Rey, A. D., Sorkin, E. & Dinarello, C. A. (1986) *Science* **233**, 652–654.
14. Sundar, S. K., Becker, K. J., Cierpial, M. A., Carpenter, M. D., Rankin, L. A., Fleener, S. L., Ritchie, J. C., Simson, P. E. & Weiss, J. M. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 6398–6402.
15. Sundar, S. K., Cierpial, M. A., Kilts, C., Ritchie, J. C. & Weiss, J. M. (1990) *J. Neurosci.* **10**, 3701–3706.
16. Brown, R., Li, Z., Vriend, C. Y., Nirula, R., Janz, L., Falk, J., Nance, D. M., Dyck, D. G. & Greenberg, A. H. (1991) *Cell. Immunol.* **132**, 84–89.
17. Riley, V., Fitzmaurice, M. A. & Spackman, D. H. (1985) in *Psychoneuroimmunology*, ed. Ader, R. (Academic, New York), pp. 31–47.
18. Weiss, J. M., Simson, P. G., Hoffman, L. J., Ambrose, M. J., Cooper, S. & Webster, A. (1986) *Neuropharmacology* **25**, 367–384.
19. Fontana, A., Weber, E. & Dayer, J. M. (1984) *J. Immunol.* **133**, 1696–1698.
20. Hertz, L., Juurlink, H. J. & Szuchet, S. (1985) in *Handbook of Neurochemistry*, ed. Lajtha, A. (Plenum, New York), pp. 603–661.
21. Giulian, D., Baker, T. J., Shih, L. N. & Lachman, L. B. (1986) *J. Exp. Med.* **164**, 594–604.
22. Cannon, J. G. & Dinarello, C. A. (1985) *Science* **227**, 1247–1249.
23. Reynolds, C. W., Timonen, T. & Herberman, R. B. (1981) *J. Immunol.* **127**, 282–287.
24. Naylor, M. R., Krishnan, K. R. R., Manepalli, A. N., Ritchie, J. C., Wilson, W. H. & Carroll, B. J. (1988) *J. Clin. Endocrinol. Metab.* **67**, 404–406.
25. Murphy, M. T., Richards, D. B. & Lipton, J. M. (1983) *Science* **221**, 192–193.
26. Daynes, R. A., Robertson, B. A., Cho, B. H., Burnham, D. K. & Newton, R. (1987) *J. Immunol.* **139**, 103–109.
27. Cannon, J. G., Tatro, J. B., Reichlin, S. & Dinarello, C. A. (1986) *J. Immunol.* **137**, 2232–2236.
28. Weiss, J. M., Sundar, K. S., Cierpial, M. A. & Ritchie, J. C. (1991) *Eur. J. Pharmacol.* **192**, 177–179.
29. Perry, V. H. & Gordon, S. (1987) *J. Exp. Med.* **166**, 1138–1143.
30. Molina, J. M., Scadden, D. T., Amirault, C., Woon, A., Vannier, E., Dinarello, C. A. & Groopman, J. E. (1990) *J. Virol.* **64**, 2901–2906.
31. Dinarello, C. A., Cannon, J. G., Mier, J. W., Bernheim, H. A., Lo-Preste, G., Lynn, G. L., Love, R. N., Webb, A. C., Auron, P. E., Reuben, R. C., Rich, A., Wolff, S. M. & Putney, S. D. (1986) *J. Clin. Invest.* **77**, 1734–1739.
32. Farrar, W. L., Kilian, P. L., Ruff, M. R., Hill, J. M. & Pert, C. B. (1987) *J. Immunol.* **139**, 459–463.
33. Takao, T., Tracey, D. E., Mitchell, W. M. & De Souza, E. B. (1990) *Endocrinology* **127**, 3070–3078.
34. Ban, E., Milon, G., Prudhomme, N., Fillion, G. & Haour, F. (1991) *Neuroscience* **43**, 21–30.
35. Gallo, P., Frei, K., Rordorf, C., Lazdins, J., Tavolato, B. & Fontana, A. (1989) *J. Neuroimmunol.* **23**, 109–116.
36. Verges, B., Chavanet, P., Desgres, J., Vaillant, G., Waldner, A., Brun, J. M. & Putelat, R. (1989) *Acta Endocrinol.* **121**, 633–637.
37. Sapolsky, R. M. (1985) *J. Neurosci.* **5**, 1228–1232.
38. McEwen, B. S., Weiss, J. M. & Schwartz, L. (1968) *Nature (London)* **220**, 911–912.
39. McEwen, B. S. & Weiss, J. M. (1970) in *Progress in Brain Research*, eds. DeWied, D. & Weijnen, J. A. W. M. (Elsevier, Amsterdam), pp. 200–212.
40. deKloet, J. M., Ratka, A., Reuk, J. M. H. M., Staano, W. & Van Eekelan, J. A. M. (1987) *Ann. N.Y. Acad. Sci.* **512**, 351–361.
41. Price, R. W., Brew, B. J. & Rosenblum, M. (1990) in *Immunologic Mechanisms in Neurologic and Psychiatric Disease*, ed. Waksman, B. H. (Raven, New York), pp. 269–290.