Intrahypothalamic Injection of the HIV-1 Envelope Glycoprotein Induces Fever via Interaction with the Chemokine System

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ABSTRACT

Wasting syndrome is a common complication of HIV infection and is marked by progressive weight loss and weakness, often associated with fever. The mechanisms involved in the pathogenesis of these syndromes are not well defined, and neither are the brain areas involved. The present study tests a new hypothesis: that the preoptic anterior hypothalamus (POAH), the main brain area for thermoregulation and fever, has a role in the pathogenesis of fever induced by glycoprotein 120 (gp120), the surface envelope protein used by the HIV to gain access into immune cells, and that the CXC chemokine receptors (CXCR4) that serve as a coreceptor for HIV entry mediate the effect. A sterilized stainless steel C313G cannula guide was implanted into the POAH, and a biotelemetry system was used to monitor

the body temperature (T_b) changes. The administration of gp120 into the POAH induced fever in a dose-dependent manner. To demonstrate possible links between the gp120 and CXCR4 in generating the fever, we pretreated the rats with 1,1'-[1,4-phenylenebis(methylene)]bis[1,4,8,11-tetraazacyclotetradecane] octahydrobromide dihydrate (AMD 3100), an antagonist of stromal cell-derived growth factor (SDF)-1 α /CXCL12, acting at its receptor, CXCR4, 30 min before administration of gp120. AMD 3100 significantly reduced the gp120-induced fever. The present studies show that the presence of HIV-1 envelope glycoprotein gp120 in the POAH provokes fever via interaction CXCR4 pathway.

The effects of HIV infection on neuronal systems are associated with the entry of the virus into the brain, which occurs soon after the initial infection (Nathanson et al., 1994). Initial events of HIV-1 cell infection involve the sequential binding of the viral envelope glycoprotein (gp120) to the cellular CD4 receptor and the coreceptor. The coreceptors are chemokine receptors; for monocytes, the coreceptor is CCR5, and for T cells, it is CXCR4. Different strains of the HIV-1 envelope glycoprotein (gp120) exhibit affinity for specific chemokine receptors differentially expressed on certain cell types. For example, gp120 associated with M-tropic HIV binds specifically to CCR5, which is expressed on macrophages, whereas gp120 associated with T-tropic HIV binds to CXCR4, which is abundant in T cells (Moore et al., 2004). A direct toxic effect on cultured hippocampal neurons by gp120 has been shown (Meucci et al., 1998). Kanmogne et al. (2007) have recently reported that HIV-1 gp120 compromises the integrity of the

blood-brain barrier. Kimes et al. (1991) have found that intracerebroventricular administration of gp120 in rats produces brain pathology. Wasting syndrome is a common complication of HIV infection and is marked by progressive weight loss and weakness, often associated with fever (Weinroth et al., 1995). It was demonstrated that brain gp120 induces a sickness behavior syndrome in rats. Indeed, the intracerebroventricular administration of gp120 produces several neurobehavioral alterations in rats, including anorexia, body weight loss, and fever (Barak et al., 2002). Fever is one of the most basic symptoms that appear in various pathological situations, and therefore the mechanism that produces fever has been an important medical topic throughout history (Kluger, 1991). It is a highly complex process initiated by the action on the brain thermosensitive cells of a number of endogenous pyrogens, which are produced by the host in response to infectious as well as noninfectious inflammatory insults (Blatteis and Sehic, 1997). The preoptic anterior hypothalamus (POAH) is generally considered to be the primary site for central control of body temperature (T_b) (Boulant et al., 1989). It contains neurons that are sensitive to subtle changes in the hypothalamus or in the body. Pre-

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ABBREVIATIONS: CCR, CC chemokine receptor; CXCR, CXC chemokine receptor; gp120, glycoprotein 120; POAH, the preoptic anterior hypothalamus; T_b , body temperature; SDF, stromal cell-derived growth factor; AMD 3100, 1,1'-[1,4-phenylenebis(methylene)]bis[1,4,8,11-tetraazacyclotetradecane] octahydrobromide dihydrate.

optic thermosensitive neurons also receive a wealth of somatosensory input from skin and spinal thermoreceptors. Fever production is controlled by neuronal systems in the brain, particularly in the POAH (Boulant, 2000). It is believed to be caused by the synthesis and release from monocytes and macrophages of a number of well characterized endogenous pyrogenic factors, including interleukin-1, interleukin-6, tumor necrosis factor- α , and macrophage inflammatory protein-1 (Myers et al., 1994; Miñano et al., 1996; Blatteis, 2006).

Chemokines are a family of small (8–12 kDa) proteins involved in cellular migration and intercellular communication. One of the chemokines thought to have an important role in the brain is SDF-1 α /CXCL12. CXCR4 and its ligand SDF-1 α /CXCL12, are constitutively expressed by glial and neuronal cells in the central nervous system (Bajetto et al., 2001). Although CXCR4 is considered the major functional receptor for CXCL12 (Bleul et al., 1996), CXCR7 has been identified as a second receptor for SDF-1 α /CXCL12 (Schöne-meier et al., 2008). Deletion of either the SDF-1 α /CXCL12 or CXCR4 gene results in abnormal cerebellar and hippocampal development, suggesting a role of this chemokinergic system in neurogenesis (Zou et al., 1998). Besides the regulation of homeostatic processes, increasing evidence implicates the SDF-1 α /CXCL12 signaling system in the pathogenesis of tumors, infections, and inflammatory processes in several diseases. CXCR4 is up-regulated in HIV and simian immunodeficiency virus encephalitis and experimental allergic encephalitis in which its expression is increased in astrocytes, infiltrating leukocytes, and/or endothelial cells on neovessels (Jiang et al., 1998; Sanders et al., 1998; Westmoreland et al., 1998). In addition, CXCR4 also act as cellular binding sites for the HIV-1 coat protein gp120, allowing the virus to interact with and infect target cells (Horuk, 1999). In the present study, we test a new hypothesis that the main brain area implicated in thermoregulation and fever is involved in the pathogenesis of fever induced by gp120 and that the chemokine system and gp120 interact to generate fever.

Materials and Methods

Animals. All animal use procedures were conducted in strict accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* (Institute of Laboratory Animal Resources, 1996) and were approved by the Institutional Animal Care and Use Committee. Young adult male Sprague-Dawley rats weighing 200 to 250 g were used in this study. They were housed three per cage for at least 1 week before surgery and were fed laboratory chow and water ad libitum. Ambient temperature was $21 \pm 0.3^\circ\text{C}$, and a 12-h light/dark cycle was used.

Surgery Procedures. Rats were anesthetized with an intraperitoneal injection of a mixture of ketamine hydrochloride (80 mg/kg) and acepromazine maleate (0.2 mg/kg). An incision 2 cm in length was made along the linea alba, and the underlying tissue was dissected and retracted. A calibrated transmitter (E-mitters, series 4000; Mini Mitter, Sunriver, OR) was then inserted in the intraperitoneal space. After the transmitter was passed through the incision, the abdominal musculature and dermis were sutured independently. For the microinjection study, a sterilized stainless steel C313G cannula guide (22-gauge; Plastics One Inc., Roanoke, VA) was implanted bilaterally in the POAH according to standard procedures (Benamar et al., 2004). The stereotaxic coordinates for the POAH implantation of guide cannula were as follows: 0.3 mm anterior to bregma, 0.5 mm from midline, and 7 mm ventral to the dura mater for POAH (Paxi-

nos and Watson, 1998). A C313DC cannula dummy (Plastics One Inc.) of identical length was inserted into the guide tube to prevent its occlusion. The animals were returned to individual cages in the environmental room.

Microinjection. After a 7-day recovery period, rats were allowed to habituate to test chambers for 1 h before testing. Either vehicle or drug was microinjected into the POAH in a volume of 0.5 μl via a C313I internal cannula (28-gauge; Plastics One Inc.). The C313I internal cannula was connected by polyethylene tubing to a 10- μl Hamilton syringe. A volume of 0.5 μl of drug or vehicle was delivered at a rate of 0.5 $\mu\text{l}/\text{min}$ (manually), and the internal cannula was left in place an additional 90 s to allow diffusion. Immediately thereafter, a dummy cannula (C313DC) was inserted into the cannula guide to prevent any contamination.

Body Temperature Measurement. Tb was measured by a biotelemetry system (Mini Mitter) using calibrated transmitters implanted intraperitoneally. Signals from the transmitter were delivered through a computer-linked receiver. This method minimizes stress to animals during the Tb reading. Thus, the Tb could be monitored continuously and recorded without restraint or any disturbance to the animal. Rats were tested in an environmental room (Hotpack, Warminster, PA), maintained at $21 \pm 0.3^\circ\text{C}$ ambient temperature and $50 \pm 2\%$ relative humidity. After 1 h of adaptation, two readings at 15-min intervals were averaged to determine the baseline. All experiments were started between 9:00 and 10:00 AM to minimize the effect of circadian variation in Tb.

Drugs. HIV-1_{MN} gp120 Recombinant Viral Protein (Advanced Biotechnologies, Columbia, MD) was used. The protein, produced using the baculovirus expression system and purified by immunoaffinity chromatography, has a molecular mass of 120 kDa. AMD 3100 was obtained from Sigma-Aldrich (St. Louis, MO) and dissolved in pyrogen-free saline.

Statistical and Histological Analysis. All data are reported as means $\pm$ S.E.M., and the variations in Tb were compared across treatments and time points and analyzed by two-way analysis of variance followed by the Bonferroni test or *t* test. The data were analyzed by Prism software (GraphPad Software Inc., San Diego, CA). Significance was set at $P < 0.05$.

At the conclusion of the experiments, each rat was injected with 0.5 μl of cresyl violet, anesthetized, and perfused transcardially with 0.9% isotonic saline, followed by phosphate-buffered saline-4% paraformaldehyde (pH 7.4). The brain was removed, stored in the same fixative for 4 h, kept in 20% sucrose overnight, and cut into 20- μm sections on a freezing microtome. Each coronal section was mounted according to standard histological procedures (Benamar et al., 2004), and the site of injection was verified by locating the dye.

Results

Effect of Intra-POAH Injection of gp120 on Tb. We first examined the action of gp120 injected into the POAH on the Tb of the rat (Fig. 1). After a 60-min baseline interval, gp120, heat-inactivated gp120, or vehicle was injected into the POAH at time 0, and Tb was measured for 300 min. All doses of gp120 tested from 33 to 133 ng caused a Tb rise after 90 min, reaching a peak elevation within 3 h after injection; this time course remained constant at all doses tested. After injection of gp120 at 33 ng, Tb was elevated significantly ($0.88 \pm 0.28^\circ\text{C}$) above baseline compared with the control, heated gp120 ($F_{4,28} = 2.71, P < 0.001$) (Fig. 1). At 66 ng gp120 produced fever within 3 h after injection, with a peak elevation of $1.1 \pm 0.16^\circ\text{C}$ ($F_{3,40} = 2.84, P < 0.001$) (Fig. 1). At 133 ng, gp120 produced the most intense fever, with a maximum of increase of $1.33 \pm 0.19^\circ\text{C}$ at 3 h after injection ($F_{4,28} = 2.71, P < 0.001$) (Fig. 1). The administration of heat-inactivated gp120 (90°C for 30 min) directly into the POAH at a

Fig. 1. Effect of intra-POAH injection of gp120 (33–133 ng) on Tb. gp120 was injected at time 0. Data are expressed as the mean $\pm$ S.E.M. from baseline. Basal body temperatures before treatment of each group was as follows: $\blacktriangle$, $37.45 \pm 0.12^\circ\text{C}$; $\blacksquare$, $37.51 \pm 0.14^\circ\text{C}$; $\blacktriangledown$, $37.37 \pm 0.10^\circ\text{C}$; $\bullet$, $37.47 \pm 0.16^\circ\text{C}$. ***, $p < 0.001$. N, number of rats.

dose of 133 ng did not induce any change in Tb compared with vehicle (0.5 μl of artificial cerebrospinal fluid).

Effect of Intra-POAH Injection of AMD 3100 on gp120-Induced Fever. To demonstrate the possible links between the gp120 and CXCR4 in the generation of fever, we pretreated the rats 30 min before gp120 with AMD 3100, an antagonist of SDF-1 α /CXCL12 at CXCR4 (Schols et al., 1997), and monitored the Tb. Doses ranging from 1.5 to 5 μg alone did not induce any significant changes in Tb compared with vehicle (Fig. 2). However, higher doses induced a significant decrease in Tb (data not shown). Accordingly, we used 5 μg of AMD 3100 to allow a clear analysis of its effects on gp120-induced fever. The administration of 5 μg significantly reduced the gp120-induced fever particularly during the 180-min postinjection compared with the vehicle/gp120 (133 ng) group ($P < 0.05$) (Fig. 3). A composite anatomical map of some individual sites of microinjection is presented in Fig. 4. The tips of the injector needles were distributed in coronal planes anterior-posterior -0.26, -0.40, -0.92, and -1.40 from bregma (Paxinos and Watson, 1998).

Discussion

The present studies are the first to demonstrate that the presence of gp120 in the brain induced fever via interaction with the chemokine system. It was previously shown that in addition to its behavioral effects, gp120 infused intracerebroventricularly also induced fever in rats (Barak et al., 2002). This effect was particularly evident during the initial hours after gp120 administration (3–5 h after injection). However, the main brain area involved in the pathogenesis of fever induced by gp120 was unknown. Because it is crucial to localize the site of action to investigate the mechanism involved, we administered the gp120 directly into the POAH

Fig. 2. Effect of intra-POAH injection AMD 3100 (1.5–5 μg) on Tb. AMD 3100 was injected at time 0. Data are expressed as the mean $\pm$ S.E.M. from baseline. Basal body temperatures before treatment of each group was as follows: $\blacktriangle$, $37.37 \pm 0.15^\circ\text{C}$; $\blacksquare$, $37.51 \pm 0.18^\circ\text{C}$; $\blacktriangledown$, $37.43 \pm 0.12^\circ\text{C}$; $\bullet$, $37.39 \pm 0.18^\circ\text{C}$. N, number of rats.

Fig. 3. Effect of intra-POAH with AMD 3100 (5 μg , -30 min) on gp120-induced fever. gp120 was injected at time 0. Data are expressed as the mean $\pm$ S.E.M. from baseline. Basal body temperatures before treatment of each group was as follows: $\blacksquare$, $37.29 \pm 0.18^\circ\text{C}$; $\bullet$, $37.47 \pm 0.17^\circ\text{C}$. *, $p < 0.05$. N, number of rats.

and used a biotelemetry system to monitor the changes in Tb. Our present data show that gp120 given directly into the POAH was able to induce a significant increase in Tb in a dose-related manner. The onset, latency, magnitude of elevation, and time course of the increase in Tb after the administration of gp120, as well as the marked sickness behavior syndrome associated with the elevation of Tb (Barak et al., 2002) are characteristic of a febrile response. It is notable that the febrile response to gp120 given intracerebroven-

Fig. 4. A, anatomical mapping in successive frontal section illustrating the distribution of some individual sites of microinjection in the POAH. B, photomicrograph showing an injection site. oc, optic chiasma; LV, lateral ventricle; ca, anterior commissure; cp, caudate putamen.

tricularly (Barak et al., 2002), in terms of onset and magnitude, was essentially concordant with our present data. However, in addition to route of injection, an important difference between that report and our present data is the dose administered. The dose we used is 20 times lower than that given by Barak et al. (2002), indicating that the POAH is highly sensitive to gp120. The gp120 effect on Tb is not due to a nonspecific interaction with hydrophobic regions of functional proteins, their lipid surroundings in the cell membrane, or contamination, because heat inactivation of this compound did not alter Tb.

The involvement of CXCR4 and SDF-1 α /CXCL12 in the HIV life cycle has generated widespread interest in the comprehensive biochemical and pharmacological characterization of this receptor. Thus, another goal of the current research was to determine the role of CXCR4 in the pathogenesis of fever induced by gp120. In situ hybridization and immunocytochemistry showed that CXCR4 neuronal expression was found mainly in several brain areas including supraoptic, paraventricular hypothalamic nuclei (Banisadr et al., 2002) and lateral hypothalamus (Guyon et al., 2005), raising the possibility that these receptors may play a role in the pathogenesis of fever induced by gp120. Davis et al. (1997) have demonstrated in cell cultures that gp120 binds to CXCR4 and can activate a signaling pathway. It was found that AMD 3100 interacts with and blocks CXCR4, the main coreceptor used by T-tropic viruses (Schols et al., 1997). In previous studies AMD 3100 was shown to inhibit the replication of T-tropic HIV strains or clinical isolates in T cell lines (such as MT-4, MOLT-4, or CEM cells) (de Clercq, 1994; De Vreese et al., 1996). In addition, the interaction between SDF-1 α /CXCL12 and CXCR4 can be blocked by AMD 3100 (Schols et al., 1997). One difficulty in studying gp120 is that it binds to more than one receptor. Understanding how cells respond to gp120 is difficult because of this promiscuity. In an attempt to establish the contribution of CXCR4 to the febrile response of gp120, AMD 3100 was administered directly into the POAH before gp120. Our present data show that AMD 3100 attenuated significantly the gp120-induced fever, indicating that CXCR4 receptors in the POAH contribute to the pathogenesis of fever induced by gp120. The effect of gp120 on Tb could occur in one of two ways. The most direct mechanism is for gp120 to bind directly to the chemokine receptors on the

neurons and thereby affect the induction of fever. The alternative mechanism is for gp120 to bind to microglia and release chemokines or other mediators of fever. Although this study was not designed to illuminate which of these two pathways is in operation, it demonstrates conclusively that this viral product interacts with CXCR4 into the POAH to generate the fever.

gp120 also binds to CCR5, a receptor that has been shown to be involved in the pathogenesis of fever induced by bacterial lipopolysaccharide (Machado et al., 2007), and the POAH administration of the ligands for this receptor [i.e., regulated on activation normal T cell expressed and secreted or macrophage inflammatory protein-1 β] has been reported to cause pyrogenesis (Davatelis et al., 1989; Tavares and Miñano, 2000). The possibility exists that CCR5 also may contribute to the effect of gp120 on Tb and may explain the lack of complete antagonism by AMD 3100.

In summary, the present data show that the presence of gp120 in the POAH provokes fever via interaction CXCR4 pathway. It seems that targeting CXCR4 may have a potential therapeutic application in interventions seeking to prevent or control the generation of fever associated with HIV-wasting syndrome.

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