Lack of Developmental Neurotoxicity of MN rgp120/HIV-1 Administered Subcutaneously to Neonatal Rats

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The potential for neurotoxic effects was evaluated in rat offspring after exposure in utero and/or during the neonatal period to a recombinant subunit vaccine of gp120 prepared from the MN strain of HIV-1 (MN rgp120/HIV-1). Thirty pregnant female rats were given MN rgp120/HIV-1 with alum adjuvant, and 30 rats were given vehicle, once every 3 days from Day 1 of presumed gestation until parturition. One pup/sex/litter from treated and control group dams were given a daily subcutaneous injection, from Day 1 through Day 22 postpartum (PP) of vehicle, MN rgp120/HIV-1, MN rgp120/HIV-1 with alum, or MN rgp120/HIV-1 with QS-21 adjuvant. Neurobehavioral and physical development were evaluated (preweaning reflex and development, sexual maturation, motor activity, acoustic startle, passive avoidance, functional observational battery, and water M-maze testing), and tissues were processed for anatomical examination (whole and regional brain weights, and neuropathology). Administration of MN rgp120/HIV-1, with or without adjuvant, to pups did not cause any persistent effect on any parameter evaluated. Neurohistological examination did not reveal any pathological effects related to treatment. Thus, MN rgp120/HIV-1 alone or formulated as a vaccine does not cause neurotoxicity or developmental toxicity in neonatal rats after exposure in utero and/or during the neonatal period.

Key Words: gp120; neurotoxicity; HIV; developmental toxicity; behavioral toxicity; QS-21.

Infection by HIV-1 is often complicated by a variety of neurological abnormalities, which include non-specific illnesses such as encephalopathies or a syndrome unique to HIV-1 infection, the AIDS dementia complex. Approximately 15–20% of AIDS patients develop this syndrome with progressive cognitive decline, motor dysfunction, and behavioral abnormalities (Brenneman et al., 1990; Power and Johnson, 1995). HIV-1 levels in dementia patients are high and sustained, while they are much lower or absent in the brains of subjects without neurologic impairment (Wiley and Achim, 1994). Several different mechanisms of neuropathogenesis are suspected, including opportunistic infections, cytokine release, intrathecal synthesis of antibody against HIV-1, and toxic products produced from the virus (Johnson, 1994).

A significant alteration of cytokine release has been observed in HIV-positive patients (Sinicco et al., 1993), and potential AIDS vaccines such as recombinant gp120 induced IFN-γ and IFN-α production in peripheral mononuclear cells from healthy donors (Capobianchi et al., 1993). In addition, 3 prominent human brain proteins have sequence homology to the V3 loop of gp120; thus, antibodies generated to a gp120 vaccine may cause an autoimmune response in the brain leading to pathogenesis (Trujillo et al., 1993). In humans, cerebrospinal fluid (CSF) samples from HIV-infected individuals have neurotoxic activity; an antiserum previously shown to bind gp120 and neutralize both infectivity and direct gp120 neurotoxicity neutralized the CSF factor (Buzy et al., 1992). Further evidence has suggested that gp120 is responsible for many of the neurotoxic effects, as neither astrocytes, oligodendrocytes, or neurons are infected with HIV-1 in vivo (Benos et al., 1994). gp120 is shed from infected cells and so has the potential to diffuse and interact with distant uninfected brain cells. Thus, AIDS vaccines which include gp120 may potentially be neurotoxic.

Several studies have shown gp120 to be neurotoxic in vitro (Apostolski et al., 1994, 1993; Kaiser et al., 1990). Treatment of human brain tissue with recombinant gp120 (SF2 and IIIB strain) did not cause neuronal death, but did cause astrocyte alterations and/or death, with decreased expression of glial fibrillary acidic protein (Pulliam et al., 1993). In vivo studies have also shown evidence of neurotoxicity caused by gp120. Transgenic mice which produce gp120 in the brain have a spectrum of neuronal and glial changes resembling abnormalities seen in brains of HIV-infected humans (Berrada et al., 1995; Toggas et al., 1994). Native (RF2 strain) and recombinant (SF strain) gp120 were administered into the cerebral ventricles of adult rats (12 ng) and performance was evaluated in the Morris swim maze. Gp120 treatment caused memory impairment as evidenced by an increase in the latency to reach
HIV-infected infants and children suffering from AIDS dementia complex may respond differently from adults. The most profound manifestation in children includes a progressive loss of previously achieved developmental milestones (Michaels and Gallo, 1994). Several studies have indicated that gp120 produces neuronal deficits in neonatal animals. Purified gp120 preparations (from the LAV, IIIB and RF2 strains of HIV-1) produced neuronal cell death in developing hippocampal cultures derived from murine fetuses at concentrations less than 1 pM (Brenneman et al., 1990).

Histological examination of the brains of rats administered intracerebroventricular gp120 on a daily basis for 3 weeks after birth revealed neuronal degeneration, dystrophic neurons, and “blebbing” of neurites (Pert et al., 1989). gp120 administered into the hippocampus of 7-day-old rats augmented the damage caused by hypoglycemia or ischemia (Barks et al., 1995), or by coinjection with NMDA (Barks et al., 1997). In vivo autoradiography of newborn rat pups which had received a scubcutaneous injection of [125I]gp120 and were killed after 1 h, revealed that radiolabeled material had reached the ventricles of the brain and diffused into the surrounding nervous tissue, and this material induced significant neuronal cell killing in a spinal cord culture assay (Hill et al., 1993). These authors also showed that 5 ng of purified gp120 (RF2 strain), administered daily to Sprague-Dawley rats by scubcutaneous (sc) injection, from birth until Day 28, significantly reduced the dendritic extent of pyramidal neurons. In addition, several developmental behaviors were delayed in gp120-treated animals, including surface righting, negative geotaxis, forelimb placing, hindlimb placing, air righting, ear twitch, and forelimb grasp.

A recombinant subunit vaccine consisting of gp120 prepared from the MN strain of HIV-1 (MN rgp120/HIV-1) is currently being evaluated as a potential AIDS vaccine in high-risk adults, in pregnant HIV-1+ females, and in children born of HIV+ mothers. This vaccine is capable of eliciting neutralizing antibody activity in rodents (Lasky et al., 1986), nonhuman primates (Berman et al., 1988), and humans (Belshé et al., 1994; Schwartz et al., 1993), and protecting chimpanzees from HIV-1 infection (Berman et al., 1988, 1990, 1996). MN rgp120/HIV-1 is currently formulated with alum, since alum is the only adjuvant used in approved vaccines in the U.S. However, alum is not active with every antigen and stimulates mostly humoral immunity (Audibert and Lise, 1993). Therefore, a purified fraction of Quil-A (saponin extracted from Quillaja saponaria), QS-21 (Kensil et al., 1991), is also currently being evaluated as an adjuvant for several vaccines (Clark et al., 1991 including MN rgp120/HIV-1.

Because of the neuropathic effects of gp120, and reports that sc injection of gp120 to neonatal rats can cause neurotoxicity and developmental toxicity (Hill et al., 1993), this study was undertaken to determine if sc injection of our MN rgp120/HIV-1 vaccine to neonatal rats from Day 1 to Day 22 postpartum caused neurotoxicity. In addition, this study was designed to determine if exposure to MN rgp120/HIV-1 in utero and/or neonatally, impaired behavioral development in these pups.

**MATERIALS AND METHODS**

**Materials.** MN rgp120/HIV-1 is produced in a genetically modified Chinese hamster ovary (CHO) cell line. The concentration of MN rgp120/HIV-1 administered to the dams was 300 µg adsorbed to aluminum hydroxide adjuvant (600 µg alum) with thimerosal added as a preservative. Vehicle consisted of tris-buffered saline with thimerosal.

Pups were treated with 100 µg/mL MN rgp120/HIV-1, 100 µg/mL MN rgp120/HIV-1 formulated with 200 µg/mL alum, or 100 µg/mL MN rgp120/HIV-1 formulated with 33 µg/mL QS-21. Stability analysis was performed, and confirmed that the material was stable throughout the conduct of the study.

**Procedure.** Sixty female Sprague Dawley rats (Charles River Laboratories, Inc., Portage, MI) were given a sc injection of 1 mL of vehicle or MN rgp120/HIV-1/alum every 3 days from Day 1 of presumed gestation (Day 0, day of mating) until parturition. A table of random units was used to select pups to be culled on postpartum (PP) Day 1, and litters were reduced to 8 pups each (4 male and 4 female). Pups (N, 30 per group from each maternal group) were randomly assigned to 4 pug treatment groups (1 pup/sex/litter for each group) as follows: (1) vehicle; (2) MN rgp120/HIV-1 in vehicle; (3) MN rgp120/HIV-1 with alum adjuvant; and (4) MN rgp120/HIV-1 with QS-21 adjuvant. Pups were given sc injections at a volume of 5 mL/kg once daily from Day 1 through Day 22 PP. Doses were selected on the basis of a pilot evaluation, where the maximum dose of 500 µg/kg/day MN rgp120/HIV-1 had no adverse effects on the pups. The maximum dose of QS-21 alone (500 µg/kg/day) caused a decrease in body weight and body weight gain. Therefore, the lower dose of 166 µg/kg/day was chosen, which maintained the ratio previously used with the dose of MN rgp120/HIV-1. This dose of MN rgp120/HIV-1 also provides approximately 100% the dose used in a similar study (Hill et al., 1993), and is approximately 20× greater than the dose given in clinical trials with MN rgp120/HIV-1 in infants born of HIV+ mothers. The sc route was chosen because dosages could be accurately administered daily in neonatal rats, and for comparison with published studies (Hill et al., 1993).

This study was conducted in compliance with U.S. FDA Good Laboratory Practice Regulations (21 CFR Part 58, 1979).

**Parameters evaluated.** Pups were evaluated for neurohistological, neurochemical, developmental, and behavioral effects in response to exposure to MN rgp120/HIV-1 in utero and/or during the neonatal period. The pups from 20 litters each from treated and control group dams were evaluated for sexual maturation, motor activity, auditory startle habituation, learning and memory (passive avoidance task and water M-maze swim test), and neurological and neurobehavioral function (functional observational battery). Ten of these 20 litters each from treated and control group dams were randomly selected for perfusion in situ, gross dissection of nervous system tissues, and measurement of regional brain weights after the behavioral testing. Five of these 10 litters were randomly selected for further processing for neurohistological examination after the behavioral testing. The remaining 10 litters from treated and control dams were sacrificed on Day 12 PP for neurohistological examination.

 Reflex and physical development parameters were monitored during the first 25 days PP (Tesch, 1997). Surface righting reflex, pinna unfolding, negative geotaxis, forelimb placing, eye opening, acoustic startle response and pinna reflex, air righting reflex, and hindlimb placing were monitored beginning on Days 1, 2, 7, 8, 12, 13, and 14 PP, respectively, until all pups in the litter reached the criterion for the specific test. Forelimb grasp and pupil reflex were measured once on Day 21 PP. Sexual maturation was monitored daily to identify prepubertal separation in males (from Day 35 PP until observed) or vaginal patency in females (from Day 28 PP until observed).

Pups were evaluated for motor activity on Days 14, 18, 22, and 61 (± 2) PP (Foss, 1994). Movements of each rat were monitored by a passive infrared sensor (Coulbourn Instruments, Lehigh Valley, PA) mounted outside a stain-
O'Donoghue, 1989) was conducted on Days 42 (±2) PP. The latency (in s) of each trial were recorded. Six trials per rat per day were given. The number of trials to reach criterion and the lighted chamber for 60 s on each of two consecutive trials. A maximum of 15 trials per rat per day were given. The number of trials to reach criterion and the latency (in s) of each trial were recorded.

Auditory startle habituation was evaluated on Days 23 and 61 (±2) PP; rats were tested for reactivity to auditory stimuli and habituation of responses with repeated presentation of stimuli (Foss and Riley, 1991). Rats were tested in sets of 4 (1 rat per dosage group of the same sex and litter) within an unlit, sound-attenuated chamber in the presence of a continuous background noise (70 dB sound level, 500 to 2000 Hz bandpass); each rat was placed in a small cage situated above a platform containing a force transducer in its base (Coulbourn Instruments, Lehigh Valley, PA). The rats were given an adaptation period of 5 min with 10 blank trials, followed by 50 trials with a 20 ms 120 dB burst of noise at 10 s intervals. The average response magnitudes and the patterns of responses across trials were compared among groups.

Passive avoidance testing was used to evaluate learning, short-term retention, long-term retention, and response inhibition (Lochry and Riley, 1980). Each rat was tested in 2-day sessions, separated by a 1-week interval, beginning on Day 24 PP. The rat was placed into the illuminated chamber of the apparatus and timed until it entered the unlit chamber; the door between the 2 chambers was then shut and a 1-s pulse of mild electric current (1 mA) delivered to the grid floor. The rat was removed from the apparatus and placed in a holding cage for 30 s. Trials were repeated until the rat remained in the lighted chamber for 60 s on each of two consecutive trials. A maximum of 15 trials per rat per day were given. The number of trials to reach criterion and the latency (in s) of each trial were recorded.

A functional observational battery (FOB) (Haggerty, 1989; Moser, 1989; O’Donohue, 1989) was conducted on Days 42 (±1) and 60 (±3) PP to assess the following parameters:

1. Lacrimation, salivation, palpebral closure, prominence of the eye, pupillary reaction to light, piloerection, respiration, urination, and defecation (autonomic functions);
2. Sensorimotor responses to visual, auditory, tactile, and painful stimuli (reactivity and sensitivity);
3. Reactions to handling and behavior in the open field (excitability);
4. Gait pattern in the open field, severity of gait abnormalities, air righting reaction, visual placing response, and landing foot splay (gait and sensorimotor coordination);
5. Forelimb and hindlimb grip strength;
6. Abnormal clinical signs including but not limited to convulsions, tremors and other unusual behavior, hypotonia or hypertonia, emaciation, dehydration, unkempt appearance, and deposits around the eyes, nose or mouth;

A water-filled, 16-gauge stainless-steel modified M-maze was used to test overt coordination, swimming ability, learning, and retention in 2 sessions separated by a 1-week interval beginning on Days 59–63 (±2) PP (Lochry et al., 1985). The concourse of the M-maze was 96.5 cm long, 20.3 cm wide and 27.9 cm high. The apparatus was filled with water to a depth of approximately 9 in and maintained at a temperature of 21 ± 1°C. The rat was placed in the base of the M-maze, at the farthest point from the 2 arms, and required to swim to the correct goal in order to be removed (the 2nd arm entered on the 1st trial of the 1st day was designated as the correct goal to control for position preference). The latency to reach the correct goal (maximum of 60 s) and the number of errors (incorrect turns in the maze) were recorded, and trials were repeated in each session until criterion was reached (either 5 consecutive, errorless trials or 15 trials completed, in that test session). Rats not achieving the correct goal within 60 sec were guided to this goal before being removed; each trial was separated by a 15-s interval.

Selected animals on Day 12, or at the completion of behavioral testing, were necropsied and tissues processed for anatomical examination [whole (PP 12, 70) and regional (PP 70) brain weights and neuropathology (PP 12, 70)]. Regional brain weights included the values for the cortex, hippocampus, striatum, olfactory bulb and tubercle, mesencephalon and diencephalon, medulla oblongata and pons, and the cerebellum. The Gasserian ganglia, the cervical, thoracic and lumbar regions of the spinal cord (with dorsal root ganglia and nerve roots), and sections of the sciatic, tibial, fibular, and sural nerves were removed and trimmed for histological processing. The central nervous system tissues, along with the ganglia and spinal nerve roots, were embedded in paraffin, and the peripheral nerves were embedded in plastic. Sections were stained with hematoxylin and eosin, luxol fast blue/cresyl violet, or toluidine blue, by the Bielschowsky’s technique.

In a separate evaluation of antibody titers, on the day of parturition 2 pups/litter were removed before nursing, decapitated, and blood samples taken. Pups from 5 litters, each from separate treated- and control-group dams, were bled retro-orbitally on Days 28 and 63 PP. In addition, on Day 14 PP, milk samples were collected from these dams, and blood samples were collected on

![FIG. 1. Body weights for pups from vehicle control (A) and MN rgp120/ HIV-1/alum-treated (B) dams. Group I, pups given vehicle; Group II, pups given MN rgp120/HIV-1 only; Group III, pups given MN rgp120/HIV-1/alum; Group IV, pups given MN rgp120/HIV-1/QS-21. Open symbols, males; filled symbols, females.](image-url)
expressed as the log₁₀ of the serum dilution which produces a reaction equal to the control. All serum and milk samples were analyzed for antibodies to MN rgp120/HIV-1. Titers are defined as the minimum possible value for positive serum.

Negative control, are prescreened at a 1/100 dilution; therefore, a titer of 2.0 twice that of a species-specific negative control serum. All sera, including the negative control, are prescreened at a 1/100 dilution; therefore, a titer of 2.0 defines the minimum possible value for positive serum.

**Statistical analysis.** Variables with interval or ratio scales of measurement were analyzed using the analysis of variance test, if the Bartlett’s test of homogeneity of variances was not significant. If the analysis of variance was significant ($p \leq 0.05$), the groups given the MN rgp120/HIV-1 were compared with the control group using Dunnett’s test. If the Bartlett’s test was significant, the Kruskal-Wallis test or Dunn’s test was used. Data from the motor activity and auditory startle habituation tests were analyzed using an analysis of variance with repeated measures.

**RESULTS**

**Dams**

There were no treatment-related effects on clinical observations, body weights, body weight gains, or feed consumption values throughout the gestation or lactation periods (data not shown).

### TABLE 1

<table>
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<th>Pup treatment</th>
<th>Sex</th>
<th>1 (Vehicle)</th>
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<th>3 (rgp120/Alum)</th>
<th>4 (rgp120/QS-21)</th>
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</table>

* rgp120, MN rgp120/HIV-1.

* Average day postpartum ± SD that the effect was observed.

* Significant from control $p \leq 0.01$.  

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**Day 22 PP.** All serum and milk samples were analyzed for antibodies to MN rgp120/HIV-1. An enzyme-linked immunosorbent assay (ELISA) was used to determine the presence and titer of antibodies to MN rgp120/HIV-1. Titers are expressed as the log₁₀ of the serum dilution which produces a reaction equal to twice that of a species-specific negative control serum. All sera, including the negative control, are prescreened at a 1/100 dilution; therefore, a titer of 2.0 defines the minimum possible value for positive serum.

**Statistical analysis.** Variables with interval or ratio scales of measurement were analyzed using the analysis of variance test, if the Bartlett’s test of homogeneity of variances was not significant. If the analysis of variance was significant ($p \leq 0.05$), the groups given the MN rgp120/HIV-1 were compared with the control group using Dunnett’s test. If the Bartlett’s test was significant, the Kruskal-Wallis test or Dunn’s test was used. Data from the motor activity and auditory startle habituation tests were analyzed using an analysis of variance with repeated measures.

**RESULTS**

**Dams**

There were no treatment-related effects on clinical observations, body weights, body weight gains, or feed consumption values throughout the gestation or lactation periods (data not shown).

### TABLE 2

<table>
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<tr>
<th>Pup treatment</th>
<th>Dosage (µg/kg/day)</th>
<th>1 (vehicle)</th>
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<th>3 (rgp120/Alum)</th>
<th>4 (rgp120/QS-21)</th>
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</table>

* rgp120, MN rgp120/HIV-1.

* M, male; F, female.

* Average day postpartum ± SD that the effect was observed.
shown). All litter evaluations were comparable between treated and control dams (data not shown). No gross lesions were attributed to administration of the MN rgp120/HIV-1 in alum adjuvant.

**Pups**

Significant numbers (p ≤ 0.01) of Group 4 (MN rgp120/HIV-1 in QS-21 adjuvant) rats from both vehicle- and MN rgp120/HIV-1-treated maternal dosage groups had sparse hair coats, usually in the area around the neck. The median onset was Day 29 PP and the median duration was 18 days. This observation did not occur in pups given the other MN rgp120/HIV-1 formulations or the vehicle control group, and was most likely due to the QS21 adjuvant. There were no significant differences in body weights (Fig. 1), body weight gains, or food consumption attributed to administration of MN rgp120/HIV-1.

There were no differences among the dosage groups in the attainment of reflex and physical developmental landmarks, except for Group 4 females which showed a delay in reaching criterion for air righting. This difference was not attributed to administration of the MN rgp120/HIV-1 formulations, because the difference was not observed in both sexes or across the different maternal dosage groups (Table 1). There were no statistically significant or biologically important differences among the groups, either in the average ages that preputial separation was first identified in the male rats, or that vaginal patency occurred in the female rats (Table 2). There were no significant differences in the FOB testing among the 4 treatment groups (data not shown).

There were no biologically important or statistically significant differences among the groups in either the number of movements or the time spent in movement when the male (Fig. 2A) and female (Fig. 2B) rats were examined in an automated motor activity apparatus on Days 22 and 61 PP. Similarly, the dosage groups had comparable auditory startle responses when that behavior was evaluated on Days 23 and 61 PP (Fig. 3). There were no differences among the groups in their performance in the passive avoidance task (Table 3), and the water M-maze task (Table 4).

There were no gross lesions observed that were attributed to administration of MN rgp120/HIV-1, and absolute brain weights and ratios of brain weight to terminal body weight were comparable in rats sacrificed on Day 12 PP and in rats sacrificed at approximately 3 months of age (Table 5). The weights of seven brain regions recorded in a subset of rats sacrificed at the end of the behavioral testing were also com-

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**FIG. 2.** Motor activity responses for male (A) and female (B) pups. Movements of each rat were monitored by a passive infrared sensor mounted outside a stainless-steel wire-bottomed cage; each test session was 1.5 h in duration. Vehicle/vehicle, pups from control dams given vehicle; vehicle/rgp120, pups from vehicle control dams given any formulation with MN rgp120/HIV-1; rgp120/vehicle, pups from MN rgp120/HIV-1/alum-treated dams given vehicle; rgp120/rgp120, pups from MN rgp120/HIV-1-treated dams given any formulation with MN rgp120/HIV-1. Filled symbols, Day 21 PP; open symbols, Day 61 PP. Average number of movements for each 10 min interval is plotted; SD for control groups only are plotted for reference.

**FIG. 3.** Average auditory startle habituation responses for male (A) and female (B) pups. Rats were tested in sets of 4 within an unlit, sound-attenuated chamber in the presence of a continuous background noise; each rat was placed in a small cage situated above a platform containing a force transducer in its base. The rats were given an adaptation period of 5 min with 10 blank trials, followed by 50 trials with a 20 ms, 120 dB burst of noise at 10 s intervals. The average response magnitudes and the patterns of responses across trials were compared among groups. Vehicle/vehicle, pups from control dams given vehicle; vehicle/rgp120, pups from vehicle control dams given any formulation with MN rgp120/HIV-1; rgp120/vehicle, pups from MN rgp120/HIV-1/alum-treated dams given vehicle; rgp120/rgp120, pups from MN rgp120/HIV-1-treated dams given any formulation with MN rgp120/HIV-1. Mean response ± SD is plotted.
Control dams

Nursing. Antibody titers were increased at Day 28 PP, and lower titers persisted at Day 63 PP. Serum titers in pups whose dams had been treated with MN rgp120/HIV-1 in alum were of approximately the same magnitude, irrespective of the treatment the pups received. For pups from the control dams, treatment with vehicle or MN rgp120/HIV-1 alone was insufficient to induce a measurable antibody titer; treatment with MN rgp120/HIV-1 in alum or MN rgp120/HIV-1 in QS-21 induced titers of 2.8 ± 0.6 and 3.8 ± 0.6, respectively, on Day 28 PP, with comparable titers maintained at Day 63 PP.

### TABLE 3
Passive Avoidance Performance of Pups

<table>
<thead>
<tr>
<th>Pup treatment</th>
<th>1 (Vehicle)</th>
<th>2 (rgp120)*</th>
<th>3 (rgp120/Alum)</th>
<th>4 (rgp120/QS-21)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dosage (μg/kg/day)</td>
<td>0</td>
<td>500</td>
<td>500/1000</td>
<td>500/166</td>
</tr>
</tbody>
</table>

Control dams

Session 1:
- Latency trial 2
  - M: 36.5 ± 19.1
  - F: 30.7 ± 15.8
- Trials to criterion
  - M: 4.6 ± 1.3
  - F: 4.7 ± 1.1

Session 2:
- Latency trial 1
  - M: 36.2 ± 20.0
  - F: 35.1 ± 21.4
- Trials to criterion
  - M: 2.8 ± 0.6
  - F: 2.7 ± 0.3

Treated dams

Session 1:
- Latency trial 2
  - M: 29.3 ± 20.1
  - F: 39.6 ± 19.2
- Trials to criterion
  - M: 5.5 ± 3.5
  - F: 5.2 ± 2.5

Session 2:
- Latency trial 1
  - M: 26.8 ± 18.8
  - F: 41.8 ± 17.6
- Trials to criterion
  - M: 2.9 ± 0.4
  - F: 3.0 ± 1.5

* rgp120, MN rgp120/HIV-1.
* Session 1 (learning phase) and Session 2 (retention phase) of testing were separated by a 1-week interval.
* M, male; F, female.

### TABLE 4
Water M-maze Performance of Pups

<table>
<thead>
<tr>
<th>Pup treatment</th>
<th>1 (Vehicle)</th>
<th>2 (rgp120)*</th>
<th>3 (rgp120/Alum)</th>
<th>4 (rgp120/QS-21)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dosage (μg/kg/day)</td>
<td>0</td>
<td>300</td>
<td>500/1000</td>
<td>500/166</td>
</tr>
</tbody>
</table>

Control dams

Session 1:
- Latency trial 2
  - M: 24.6 ± 16.3
  - F: 12.1 ± 4.7
- Trials to criterion
  - M: 7.6 ± 2.3
  - F: 7.8 ± 2.2

Session 2:
- Latency trial 1
  - M: 11.1 ± 6.4
  - F: 10.5 ± 7.0
- Trials to criterion
  - M: 6.9 ± 2.6
  - F: 7.3 ± 3.1

Treated dams

Session 1:
- Latency trial 2
  - M: 19.1 ± 11.8
  - F: 14.0 ± 7.1
- Trials to criterion
  - M: 7.7 ± 1.8
  - F: 7.6 ± 2.0

Session 2:
- Latency trial 1
  - M: 12.9 ± 6.9
  - F: 15.7 ± 13.8
- Trials to criterion
  - M: 5.6 ± 1.1
  - F: 6.8 ± 2.6

* kg = MN rgp120/HIV-1.
* Sessions 1 (learning phase) and 2 (retention phase) of testing were separated by a 1 week interval.
* M, male; F, female.

Latency to reach criterion is measured in s ± SD; Session 1 had 2 trials, Session 2 had 1 trial.
TABLE 5
Regional Brain Weights of Pups after Behavioral Testing

<table>
<thead>
<tr>
<th>Pup treatment</th>
<th>Dosage (µg/kg/day)</th>
<th>Control dams</th>
<th>Treated dams</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Male Female</td>
<td>Male Female</td>
</tr>
<tr>
<td>Brain weight</td>
<td>2.160 ± 0.06 1.940 ± 0.06</td>
<td>2.160 ± 0.07 1.970 ± 0.06</td>
<td>2.150 ± 0.11 1.980 ± 0.08</td>
</tr>
<tr>
<td>Brain (% TBW)</td>
<td>0.420 ± 0.03 0.730 ± 0.02</td>
<td>0.430 ± 0.03 0.720 ± 0.07</td>
<td>0.430 ± 0.04 0.740 ± 0.04</td>
</tr>
<tr>
<td>Cortex</td>
<td>0.913 ± 0.64 0.833 ± 0.62</td>
<td>0.902 ± 0.42 0.825 ± 0.73</td>
<td>0.903 ± 0.46 0.822 ± 0.51</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>0.128 ± 0.09 0.118 ± 0.16</td>
<td>0.129 ± 0.19 0.116 ± 0.13</td>
<td>0.130 ± 0.17 0.132 ± 0.09</td>
</tr>
<tr>
<td>Striatum</td>
<td>0.054 ± 0.11 0.056 ± 0.07</td>
<td>0.051 ± 0.13 0.055 ± 0.13</td>
<td>0.058 ± 0.09 0.052 ± 0.09</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>0.303 ± 0.14 0.272 ± 0.15</td>
<td>0.300 ± 0.23 0.279 ± 0.12</td>
<td>0.300 ± 0.20 0.273 ± 0.10</td>
</tr>
</tbody>
</table>

DISCUSSION

The present study has shown that injections of MN rgp120/HIV-1 in alum in pregnant rats every 3 days during gestation has no adverse effects on these dams or their offspring. Subsequent injections of MN rgp120/HIV-1 in QS-21 in the pups, for the first 22 days PP, caused sparse hair coats in significant numbers of these rats, beginning around the time of weaning and persisting for up to 1 month. This formulation did not cause other adverse clinical observations, nor did this or the other formulations that were administered to the pups affect survival, body weights, feed consumption values, reflex and physical development, or behavioral performance (functional observational battery, motor activity, auditory startle habituation, passive avoidance, escape in a water M-maze). No gross lesions or neuropathological alterations were considered effects of MN rgp120/HIV-1 administration, and brain weights were comparable among the dosage groups.

Thus, at doses approximately 100× over those reported previously to cause learning and behavioral deficits in rats (at 5 ng gp120 daily sc, from birth until Day 28 PP; developmental milestones were delayed, including surface righting, negative geotaxis, forelimb and hindlimb placing, air righting, ear switch, and forelimb grasp (Hill et al., 1993)), there was no evidence of neurotoxicity of the MN rgp120 vaccine. It is not evidence of neurotoxicity of the MN rgp120 vaccine. It is not

TABLE 6
Analysis of Antibody Titers in Milk and Serum from Dams and in Serum from Pups

<table>
<thead>
<tr>
<th>Dam group</th>
<th>Treatment (1 mL dose)</th>
<th>Dams Day 14 milk</th>
<th>Dams Day 22 serum</th>
<th>Pups Day 1 serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Vehicle</td>
<td>&lt; 2</td>
<td>&lt; 2</td>
<td>&lt; 2</td>
</tr>
<tr>
<td>II</td>
<td>300/600 µg MN rgp120/Alum</td>
<td>4.1 ± 0.4</td>
<td>4.8 ± 0.3</td>
<td>3.5 ± 0.3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Pup group</th>
<th>MN rgp120 µg/kg/day</th>
<th>Formulation; µg/kg/day</th>
<th>Day 28</th>
<th>Day 63</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>Vehicle</td>
<td>&lt; 2</td>
<td>&lt; 2</td>
</tr>
<tr>
<td>2</td>
<td>500</td>
<td>Vehicle</td>
<td>&lt; 2</td>
<td>&lt; 2</td>
</tr>
<tr>
<td>3</td>
<td>500</td>
<td>Alum; 1000</td>
<td>2.8 ± 0.6</td>
<td>2.5 ± 0.5</td>
</tr>
<tr>
<td>4</td>
<td>500</td>
<td>QS-21; 166</td>
<td>3.8 ± 0.8</td>
<td>2.8 ± 0.6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Dam group I</th>
<th>Dam group II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 28</td>
<td>Day 63</td>
</tr>
<tr>
<td>4.5 ± 0.3</td>
<td>3.1 ± 0.4</td>
</tr>
<tr>
<td>4.4 ± 0.4</td>
<td>2.9 ± 0.4</td>
</tr>
<tr>
<td>4.3 ± 0.4</td>
<td>2.8 ± 0.3</td>
</tr>
<tr>
<td>4.0 ± 0.2</td>
<td>3.0 ± 0.3</td>
</tr>
</tbody>
</table>

Note. In groups where not all animals seroconverted, a titer of 1.7 has been assigned to negative samples for calculation of group means and standard deviations. For pup groups, data for males and females are combined; N, 10.

*Group size, 9.
clear why the data from the current study differ from those reported previously. The strain of gp120 used in our study (MN) was different from the strain (RF2) used in the study by Hill et al. (1993), and this may have caused a difference in the neurotoxic effects seen. For example, the ability to infect brain-derived cells with HIV-1 differs depending on specific mutations in the V3 region of gp120 (Shimizu et al., 1994). In addition, Power et al. (1994) found that HIV-1 isolated from AIDS patients with dementia differed in the sequence of the V3 loop from HIV-1 isolated from patients with no dementia. Differences in the V3 loop of gp120 define which cell types the HIV-1 infects, and may alter binding to CD4 and subsequent development of neurotoxicity (Scorziello et al., 1998). Macrophage tropism is a necessary feature of an HIV strain in order to infect the CNS and subsequently cause disease, and the principal cell infected in the CNS is the microglia, which is of macrophage lineage (Power and Johnson, 1995).

Administration to dams of MN rgp120/HIV-1 in alum induced high antibody titers in both the serum and milk, which were transferred to the pups both in utero and through nursing. Pups from dams exposed to MN rgp120/HIV-1 had comparable antibody titers regardless of which treatment the pup received postnatally. Pups which were not exposed in utero developed antibody titers when treated with MN rgp120/HIV-1 in alum, and slightly higher titers when treated with MN rgp120/HIV-1 in QS-21. Thus, exposure of neonatal rats to significant titers of antibodies against rgp120 also did not cause any neurotoxicity or developmental toxicity.

HIV-1-related neuronal injury is a complicated process, with multiple, complex interactions and feedback loops participating in neurotoxicity (Lipton, 1994). gp120 may be acting directly on astrocytes to block growth factors and produce nitric oxide, which causes neuronal damage, or indirectly through production of cytokines or neurotoxins from macrophages, such as arachidonic acid metabolites and platelet activating factor, which then stimulate the increase in [Ca^{2+}], and glutamate. Although gp120 may play a role in HIV-induced neuropathies, and others have shown that gp120 does enter the brain following sc injection and cause neurotoxicity (Hill et al., 1993), there was no evidence of neurotoxicity in the present study following sc injection of high doses of MN rgp120/HIV-1. It is likely that the route of administration, the combination with adjuvant, and the amount of the recombinant version of gp120 from the MN strain of HIV-1 given as a vaccine are not sufficient to cause neurotoxicity, even in neonatal animals. However, even the MN rgp120/HIV-1 without adjuvant, given by sc administration, was not sufficient to cause neurotoxicity or developmental toxicity in these rats. Thus, data from this study suggest that the MN rgp120/HIV-1, given alone or with various adjuvants, would be safe to administer to children who are exposed in utero to both gp120 and antibodies to gp120 without causing neurotoxicity or developmental toxicity.

REFERENCES

Foss, J. A. (1994). The application of a functional observational battery and


