Journal of NeuroVirology, 11: 34–45, 2005

ISSN: 1355-0284 print / 1538-2443 online
DOI: 10.1080/13550280590901732

Taylor & Francis
Taylor & Francis Group

Cognitive and motor deficits associated with HIV-2_{287} infection in infant pigtailed macaques: A nonhuman primate model of pediatric neuro–AIDS

JM Worlein,[1] J Leigh,[1] K Larsen,[1] L Kinman,[2] A Schmidt,[1] H Ochs,[3] and RJY Ho[2]

[1]*Washington National Primate Research Center;* [2]*Department of Pharmaceutics;* [3]*Department of Pediatrics, University of Washington, Seattle, Washington, USA*

Lentivirus-infected nonhuman primates exhibit behavioral and neurological pathology similar to human immunodeficiency virus (HIV)-infected humans and offer a means to examine the effects of lentivirus infection while controlling for confounding factors inherent in human populations. The purpose of this study was to examine cognitive and motor development in infant macaques vertically infected with HIV-2_{287}. Subjects were 20 infant pigtail macaques (*Macaca nemestrina*); 8 controls born to uninfected dams, and 12 infants whose dams had been inoculated and infected with HIV-2_{287} in the third trimester of pregnancy. Eight of these pregnancies had undergone surgical procedures in the form of maternal amniotic catheters or maternal amniotic and fetal carotid artery and jugular vein catheters. Data indicated that catheterization had little or no impact on behavioral development. Seven infants were vertically infected (as measured by polymerase chain reaction (PCR) at birth) and five were not infected (as measured by PCR and coculture on repeated testing). Infected infants attained cognitive and motor milestones at significantly later ages than controls. Uninfected infants, born to infected dams, attained developmental milestones at later ages than controls on all tasks, but this reached statistical significance only for the Fine Motor Task. Attainment of milestones was not correlated with viral dose, maternal CD4+ levels at parturition or infant viral RNA levels at birth. Attainment of milestones was negatively correlated with infants' proportions of CD4+ lymphocytes at birth and significantly correlated with proportions of CD4+ lymphocytes 2 weeks after birth, indicating poorer performance in those infants with a more rapid CD4+ depletion. These cognitive and motor deficits closely resemble those observed in human infants and children infected with HIV and indicate that HIV-2_{287}–infected infant macaques represent an excellent model of pediatric neuro–acquired immunodeficiency syndrome (neuroAIDS). *Journal of NeuroVirology* (2005) **11,** 34–45.

Keywords: AIDS; animal model; cognitive development; infant; motor development; neuro-AIDS; nonhuman primate

Introduction

The acquired immunodeficiency syndrome (AIDS) pandemic continues to grow. Data from the World Health Organization (WHO) indicate that as of the end of the year 2003, there were 46 million people infected with human immunodeficiency virus (HIV). Of these, 2.9 million were infants and children under the age of 15. Central nervous system (CNS) involvement due to HIV infection is especially prevalent in the children where the brain is a primary site of HIV infection (Epstein, 1988). It is estimated that 78% to 93% of infants and children infected with HIV will show symptoms of neurological dysfunction (Belman, 1992; Belman *et al*, 1988; Epstein *et al*, 1984; Schwarcz and Rutherford, 1989). In addition, the presence of encephalopathy in children is

Address correspondence to Dr. Julie M. Worlein, Washington National Primate Research Center, University of Washington, Box 357330, Seattle, WA 98195, USA. E-mail: worleinj@bart.rprc.washington.edu

Received 27 February 2003; revised 19 July 2004; accepted 2 September 2004.

associated with poor prognosis (Epstein *et al*, 1986; Lobato *et al*, 1995; Pearson *et al*, 2000; Tovo *et al*, 1992). Encephalopathy may be the first indication of HIV infection in up to 16% of children (Angelini *et al*, 2000; Cooper *et al*, 1998; Scott *et al*, 1989; Tovo *et al*, 1992), although neurological symptoms are more likely to appear with advanced infection (Lobato *et al*, 1995; Nozyce *et al*, 1994).

Infants and children infected with HIV often show frank signs of cognitive and motor impairment. These include developmental delay and/or loss of previously acquired developmental milestones, mental retardation, and deficits of visual-spatial integrative ability and memory (Belman *et al*, 1988; Boivin *et al*, 1995; Chase *et al*, 2000; Diamond, 1989; Diamond *et al*, 1987, 1990; Drotar *et al*, 1997, 1999; Knight *et al*, 2000; Nozyce *et al*, 1994). Language abilities may also be compromised (Belman *et al*, 1988; Coplan *et al*, 1998), as well as both fine and gross motor functioning (Boivin *et al*, 1995; Chase *et al*, 2000; Knight *et al*, 2000). Motor impairments are the most common symptom seen in infants and children and vary from clumsiness and ataxia to rigidity, hypotonicity, tremor, spasticity, cerebellar signs, hyperactive deep tendon reflexes, and pyramidal tract signs (Belman, 1990; Englund *et al*, 1996). HIV-infected infants have also been shown to have problems with attention and concentration, often associated with hyperactivity or withdrawal (Lifschitz *et al*, 1989; Moss *et al*, 1989, 1990), as well as deficits in social and emotional development (Englund *et al*, 1996; Moss *et al*, 1996; Wolters *et al*, 1994). HIV-infected infants often evince delay in acquisition of social smile and children with HIV encephalopathy score significantly higher on scales of depression, autism, and irritability and are more apathetic and less social than controls (Belman, 1990; Moss *et al*, 1994). Infants who were infected prenatally score lower on measures of cognitive and motor performance than do peri/postnatally infected infants (Smith *et al*, 2001).

Although HIV causes neurologic disease in both adults and children, the manifestations of HIV-associated neurologic disease differ between adults and children in several important ways. Children show much more rapid clinical presentation than adults, who typically show a long latency from primary infection to neurologic symptoms (Mintz, 1994; Price *et al*, 1988). Children also appear to harbor a greater viral load in cerebrospinal fluid (CSF) than adults (Pratt *et al*, 1996). Secondary infections in the brain, common in adults, are rarely seen in the pediatric population (Belman *et al*, 1988; Sharer *et al*, 1986), and seizures, cerebrovascular disease, and stroke are also less common (Mintz, 1994). Thus, neurologic symptoms in the young subject may represent a better model of the specific effects of the virus on the brain. It is also important to note that CNS disease in adults represents the deterioration of a mature CNS, whereas CNS disease in infants and children manifests itself as impairment of growth of an immature CNS. It has been hypothesized that the behavioral effects of HIV are much more devastating in children due to the action of the virus on a developing nervous system (Mintz, 1998).

Behavioral studies in human infants are confounded by maternal drug use, lack of prenatal care, low socioeconomic status, and prolonged periods of hospitalization and foster care, making the study of neurobehavioral sequelae difficult (Coscia *et al*, 2001; Mellins *et al*, 1994; Wilkins *et al*, 1990). These confounding factors, inherent in human studies, can be avoided by using a nonhuman primate model. In addition, one can manipulate nonhuman primates in ways not possible in human populations, enabling controlled examination of mechanisms, effects, and possible side effects of therapies. Nonhuman primates infected with SIV evince neuropathology similar to that seen in humans (Eiden *et al*, 1993; Sharer *et al*, 1997; Fox *et al*, 1997; Westmoreland, 1999). Nonhuman primates infected with SIV also show impairments in motor and cognitive performance, abnormalities in sensory- and motor-evoked responses, and disruptions in circadian rhythms and motor activity (Fox *et al*, 1996; Gold *et al*, 1998; Horn *et al*, 1998; Marcario *et al*, 1999; Murray *et al*, 1992; Prospero-Garcia, *et al*, 1996; Rausch *et al*, 1994a, 1994b, 1995; Raymond *et al*, 1999). Nonhuman primates are especially valuable for developmental studies as their brain develops in a manner similar to human infants but the developmental process is four times faster, thus allowing developmental studies to be compressed in time.

HIV-2_{287} is a virus that has proven to be highly pathogenic in macaques. Both adult and infant *Macaca nemestrina* inoculated with HIV-2_{287} routinely develop high virus loads and rapid CD4+ cell depletion (to $< 200\ \mu$l) within a few weeks post inoculation (Herz *et al*, 2002; Ho *et al*, 1996; Looney *et al*, 1998; McClure *et al*, 2000; Watson *et al*, 1997). HIV-2_{287} was developed from the culture supernatant of the original isolate of human strain, HIV-2_{EHO}, obtained from Dr. Luc Montagnier at the Pasteur Institute. In humans, although the clinical course of HIV-2 is more prolonged when compared to HIV-1, CNS involvement may be more prevalent (Lucas *et al*, 1993). The purpose of this study was to evaluate motor and cognitive sequelae in infant pigtail macaques (*M. nemestrina)* vertically infected with HIV-2_{287} and to develop a nonhuman primate model of pediatric neuroAIDS.

Results

Hematological data

Hematological data were collected from inoculated dams and their infants. Blood samples were not available for one infected infant at birth and one noninfected infant at 2 weeks after birth. Absolute numbers

of CD4+ lymphocytes were also not available for one infected infant at birth. Dams of HIV-infected infants had lower proportions (6.6% versus 26%) and absolute numbers (95 versus 259) of CD4+ cells at the time of delivery although this did not reach statistical significance ($P > .05$). Infected infants (INFs) had lower proportions and absoulte numbers of CD4+ lymphocytes than exposed but not infected infants (EXPs) at birth (Figure 1A and B) and 2 weeks after birth (Figure 1C and D). Differences were statistically significant for absolute numbers of CD4 lymphocytes at birth ($P = .04$) and were statistically significant for both proportions ($P = .02$) and absolute numbers ($P = .01$) at the 2-week sample. Mean proportions of CD4+ lymphocytes for EXPs were within the range of normal macaque infants, but INFs were below this range (DeMaria *et al*, 2000; Terao *et al*, 1988).

Motor development

Plain reach: The analysis of variance (ANOVA) was significant ($P = .004$) for the age that infants were able to pick up a small brightly colored toy (Figure 2A). Post hoc analysis revealed that INFs were significantly older than CONs ($P = .001$) when they attained this motor milestone.

Fine motor task: The ANOVA was also significant for the age that infants were able to pick up a small piece of fruit in a pincer grasp ($P = .001$). Post hoc analysis revealed that both EPD's ($P = .01$) and INFs ($P \leq .001$) were significantly older than controls when they attained this milestone (Figure 2B). INFs were also significantly different from EPDs ($P = .04$).

Cognitive development

Screen task: The ANOVA was significant ($P = .01$) for the age infants were able to retrieve an object in the No Hide condition for the Screen Task (Figure 3A). Post hoc analysis revealed that INFs attained this developmental milestone at a significantly later age than CONs ($P = .003$). EPDs were not significantly different from either CONs or INFs. The ANOVA was also significant ($P = .004$) for the age that infants were able to recognize and retrieve an object partially hidden behind the opaque screen (Figure 3B). Post hoc analysis showed that INFs attained this developmental milestone at significantly later ages than EPDs ($P = .04$) and CONs ($P = .001$). Group effects were similar for objects in the Full Hide condition (Figure 3C), although the overall ANOVA did not reach statistical significance ($P = .09$).

Well task: The ANOVA was significant ($P = .007$) for the age infants were able to retrieve an object in the No Hide condition for the Well Task (Figure 3D). As with the screen task post hoc analysis revealed that

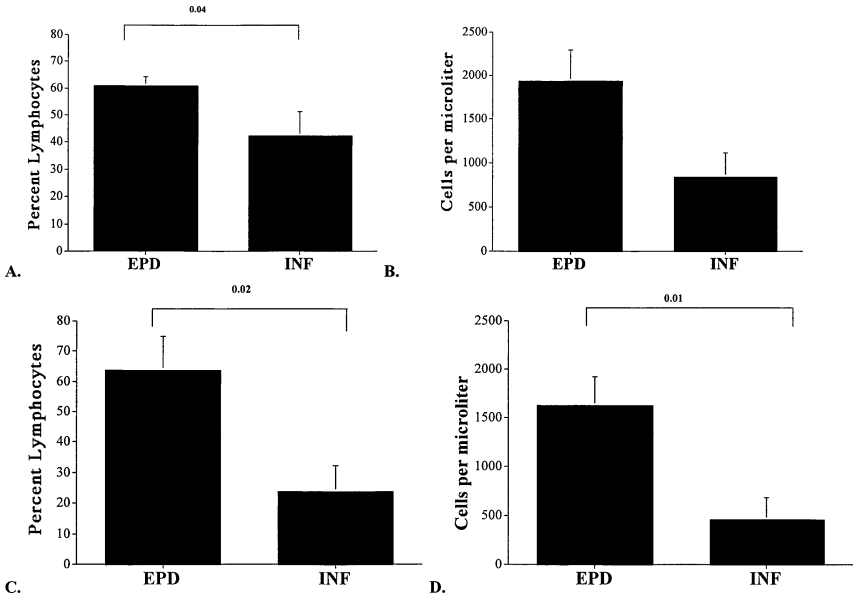

Figure 1 Proportion of CD4+ lymphocytes. (**A**) Means and standard errors of proportions of CD4+ lymphocytes for $HIV2_{287}$-exposed (EPD; $n = 5$) and $HIV2_{287}$-infected (INF; $n = 6$) infants at birth. EPD infants had higher proportions of CD4+ lymphocytes at birth; however, this difference was not statistically significant ($P = .10$). (**B**) Means and standard errors of absolute numbers of CD4+ lymphocytes at birth. EPD ($n = 5$) infants had a significantly greater number of CD4+ lymphocytes than INF ($n = 5$) infants at birth ($P = .04$). (**C**) Means and standard errors of proportions of CD4+ lymphocytes for $HIV2_{287}$-exposed (EPD; $n = 5$) and $HIV2_{287}$-infected (INF; $n = 7$) infants at 2 weeks of age. EPD infants had significantly more CD4+ lymphocytes ($P = .02$) than INF infants. (**D**) Means and standard errors of absolute numbers of CD4+ lymphocytes at 2 weeks of age. EPD infants had significantly more CD4 lymphocytes ($P = .001$) than INF infants.

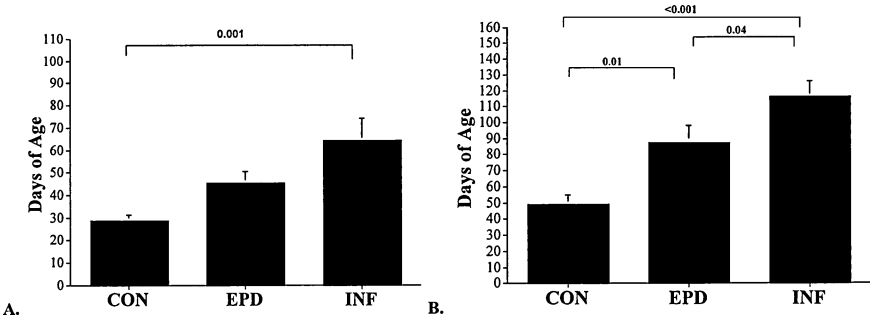

Figure 2 Motor development Tasks. (**A**) Plain Reach: Days of age that macaque infants were able to pick up a small brightly colored toy. Means and standard errors for control (CON; $n = 8$), HIV2$_{287}$-exposed (EPD; $n = 5$) and HIV2$_{287}$-infected (INF; $n = 7$) infants. The overall ANOVA was significantly among groups ($P = .004$). Post hoc tests revealed that INFs were significantly different from CONs ($P = .001$). (**B**) Fine Motor Task: Days of age that macaque infants were able to pick up a small piece of fruit in a pincer grasp. Means and standard errors for control (CON; $n = 5$), HIV2$_{287}$-exposed (EPD; $n = 5$) and HIV2$_{287}$-infected (INF; $n = 6$) infants. The overall ANOVA was significant among groups ($P = .001$). Post hoc tests indicated that INFs were significantly different from both CONs ($P < .001$) and EPDs ($P = .04$). EPDs were also significantly different from CONs ($P = .01$).

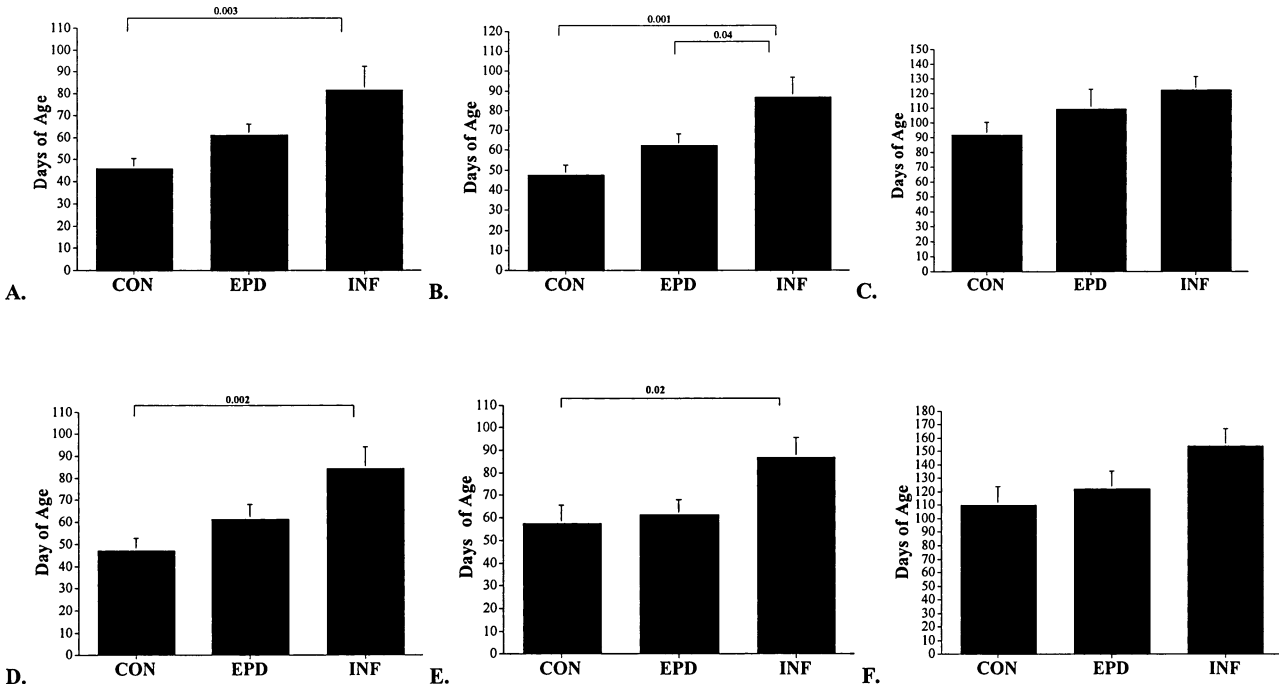

Figure 3 Cognitive development tasks: Screen Task. (**A**) No Hide: Days of age that macaque infants were able to retrieve a toy when place on a platform with an opaque screen. Means and standard errors for control (CON; $n = 8$), HIV2$_{287}$-exposed (EPD; $n = 5$) and HIV2$_{287}$-infected (INF; $n = 7$) infants. The overall ANOVA was significant among groups ($P = .01$). Post HOC tests revealed that INFs were significantly different from CONs ($P = .003$). (**B**) Partial Hide: Days of age that macaque infants were able to retrieve a toy when it was partially obscured behind an opaque screen. Means and standard errors for control (CON; $n = 8$), HIV2$_{287}$-exposed (EPD; $n = 5$) and HIV2$_{287}$-infected (INF; $n = 7$) infants. The overall ANOVA was significant among groups ($p = .001$). Post hoc tests revealed that INFs were significantly different from both EPDs ($P = .04$) and CONs ($P = .001$). (**C**) Full Hide: Days of age that macaque infants were able to retrieve a toy when it was fully hidden behind an opaque screen. Means and standard errors for control (CON; $n = 8$), HIV2$_{287}$-exposed (EPD; $n = 5$) and HIV2$_{287}$-infected (INF; $n = 7$) infants. There was a trend for differences among groups; however, the overall ANOVA not reach statistical significance ($P = .09$). Cognitive development task: Well Task (**D**) No Hide: Days of age that macaque infants were able to retrieve a toy when it was placed in a shallow well. Means and standard errors for control (CON; $n = 8$), HIV2$_{287}$-exposed (EPD; $n = 5$) and HIV2$_{287}$-infected (INF; $n = 7$) infants. The overall ANOVA was significantly different among groups ($P = .007$). Post hoc tests revealed that INFs were significantly different from CONs ($P = .002$). (**E**) Partial Hide: Days of age that macaque infants were able to retrieve a toy placed in a shallow well and partially covered by an opaque lid. Means and standard errors for control (CON; $n = 8$), HIV2$_{287}$-exposed (EPD; $n = 5$) and HIV2$_{287}$-infected (INF; $n = 7$) infants. The overall ANOVA was significant among groups ($P = .04$). Post hoc tests revealed that INFs were significantly different from CONs ($P = .02$). (**F**) Full Hide: Days of age that macaque infants were able to retrieve a toy placed in a shallow well that was totally covered by an opaque lid. Means and standard errors for control (CON; $n = 6$), HIV2$_{287}$-exposed (EPD; $n = 5$) and HIV2$_{287}$-infected (INF; $n = 6$) infants. There was a trend for differences among groups, however, the overall ANOVA did not reach statistical significance ($P = .07$).

INFs were significantly older than CONs ($P = .002$) when they attained this developmental milestone. The ANOVA was also significant ($P = .04$) for the Partial Hide condition (Figure 3E). Post hoc analysis showed that INFs were significantly older than CONs ($P = .02$) when they gained this cognitive milestone. Results were similar for objects fully hidden in the well (Figure 3F), although the ANOVA did not reach statistical significance ($P = .07$). This may be in part due to loss of power as three controls (planned euthanasia) and one infected infant (clinical euthanasia) were lost prior to completing this task.

Correlational data

Spearman's correlations (SYSTAT) were conducted comparing cognitive and motor development against lymphocyte and plasma viral RNA data obtained from the dams and infected infants. Corrections were not made for multiple correlations in this small sample. Analysis indicated that there were no consistent patterns of correlations between maternal viral dose or maternal CD4+ levels at birth and infant cognitive and motor development (data not shown). There was also no consistent pattern of correlations for infant levels of viral RNA at birth and subsequent behavioral development (data not shown). What did emerge were negative correlations for all cognitive milestones and one motor milestone (Plain Reach) with CD4+ proportions at birth. However these correlations only reached statistical significance for one measure (Full Hide Screen). Lower CD4+ proportions correlated with longer times to attain cognitive and motor milestones. These correlations became stronger for most measures when related to CD4+ proportions approximately 2 weeks after birth (Table 1). These correlations were significant for all phases of the Screen Task (No Hide, Partial Hide, and Full Hide) and for two phases of the Well Task (No Hide and Partial Hide).

Correlations among tasks

The two motor tasks (Plain Reach and Fine Motor Task) were not significantly correlated with each other or any of the cognitive measures ($P \geq .05$). There were several significant correlations among cognitive tasks (Table 2). No Screen was significantly correlated with Partial Screen, No Well, and Partial Well. No Well was significantly correlated with Partial Screen and Partial Well, and Partial Screen was significantly correlated with Partial Well ($P \leq .01$).

Table 1 Correlation among proportions of CD4+ lymphocytes and behavioral measures for $HIV2_{287}$-infected macaque infants

	CD4 % at Birth	*CD4 % at 2 weeks*
Plain Reach	−.371 ($n = 6$)	−.464 ($n = 7$)
Fine Motor Task	.100 ($n = 5$)	−.600 ($n = 6$)
No Hide Screen	−.486 ($n = 6$)	−.857^{+} ($n = 7$)
Partial Hide Screen	−.486 ($n = 6$)	−.857^{+} ($n = 7$)
Full Hide Screen	−.886* ($n = 6$)	−.857^{+} ($n = 7$)
No Hide Well	−.314 ($n = 6$)	−.821^{+} ($n = 7$)
Partial Hide Well	−.314 ($n = 6$)	−.821^{+} ($n = 7$)
Full Hide Well	−.300 ($n = 5$)	−.086 ($n = 6$)

Note. Number of subjects for each correlation is noted in parenthesis.
$^{*}P \leq .05$, $^{+}P \leq .01$, Spearman rank-order coefficient one-sided test.

Table 2 Correlation among cognitive tasks for $HIV2_{287}$-infected macaque infants

	No screen	*Partial screen*	*Full screen*	*No well*	*Partial well*	*Full well*
Partial screen	1.00^{+}					
Full screen	.679	.679				
No well	.964^{+}	.964^{+}	.607			
Partial well	.964^{+}	.964^{+}	.607	1.00^{+}		
Full well	0.00	0.00	.250	−.179	−.179	

$^{+}P \leq .01$, Spearman rank-order coefficient two-sided test.

Discussion

Data from this study clearly indicate that vertically infected HIV-2_{287} macaque infants show developmental delays that are apparent from early stages of development. Infected infants attained developmental milestones at later ages for most of the stages of Object Concept. Many of these delays are quite profound. Control infants attained the earliest cognitive milestones (No Hide conditions for Screen and Well) at approximately $1\frac{1}{2}$ months of age, whereas it took infected infants nearly twice as long to attain these milestones. In addition, infected infants were delayed in their motor abilities attaining these milestones at later ages than controls.

These deficits in cognitive and motor development are similar to those seen in human infants infected with HIV-1. For example, Chase *et al* (2000) found deficits on the Bayley scales of mental (MDI) and psychomotor (PDI) development in HIV-infected infants as young as 4 months of age. Likewise, MacMillan *et al* (2001) found deficits in the MDI and PDI at 4 months of age in HIV-infected infants and the deficits became more pronounced by 24 months of age. Belman *et al* (1996) reported deficits in both fine and gross motor domains in human infants appearing as early as 6 months of age, with fine motor skills becoming progressively worse with age. The largest differences among groups in this study appeared in the two tests of motor development, Plain Reach and Fine Motor Task. For both tests, HIV-2_{287}–infected infants were over twice the age of their uninfected counterparts when they attained these motor milestones. These data are consistent with those of HIV-infected human infants whose motor development is most strongly affected by viral infection (Drotar *et al*, 1997; Englund *et al*, 1996).

There were significant correlations among several of the cognitive tasks. This is not surprising because

milestones measured in this study are usually attained in a set developmental order. However, neither of the motor tasks was significantly correlated with any of the cognitive tasks. This disassociation of cognitive and motor deficits is also seen in human HIV-infected infants, with some infants having greater motor than cognitive impairment and others greater cognitive than motor impairment (Belman, 2002).

It is interesting to note that infants exposed to virus *in utero*, but remained HIV negative, attained developmental milestones at later ages than controls on all motor and most cognitive tasks. These differences reached statistical significance for the Fine Motor Task. Exposed but not infected human infants are commonly referred to as seroreverters. Although several studies have found no developmental delays in seroreverters (Aylward *et al*, 1992; Belman *et al*, 1996; Chase *et al*, 1995; Nozyce *et al*, 1994), performance of seroreverters routinely falls between that of infected infants and infants born to HIV-negative mothers, especially for measures of motor development (Boivin *et al*, 1995; Bruck *et al*, 2001; Drotar *et al*, 1997; Pollack *et al*, 1996).

Some studies, however, have found significant developmental differences between seroreverters and control infants born to HIV-negative mothers. For example, Esposito *et al* (1999) found that seroreverter children showed significantly poorer skills in verbal recall and had more problems in social adjustment and attention than children born to uninfected mothers. Bruck *et al* (2001) reported mild neurological abnormalities in 40% of seroreverter infants. It has also been shown that being born to a HIV-infected mother can affect certain infant hematological and immunological parameters even though the infants are not themselves infected (Chougnet *et al*, 2000; Clerici *et al*, 2000; Gesner *et al*, 1994; Kuhn *et al*, 2001, 2002; Nielsen *et al*, 2001). It is possible that behavioral differences between seroreverters and controls are not easily detectable in the human population due to the wide variety of confounding factors (such as prenatal drug use, etc.) that add to the variance of data obtained. The possible enduring effects of being born to an HIV-infected mother is of growing importance, as drug therapies decrease the chances of infants being born infected. The nonhuman primate model, which exhibits similar behavioral development and is free of maternal confounding factors, could provide an indispensable means to develop therapeutic interventions during pregnancy for the growing proportion of HIV-exposed infants who remain virus free.

Lower proportions of CD4+ lymphocytes at birth and shortly thereafter predicted poorer cognitive and motor development. These data are in agreement with Tardieu *et al* (1995) who found that CD4+ counts at an earlier time point predicted academic performance at a later age. The fact that proportions of CD4+ lymphocytes at 2 weeks were most predictive of behavioral performance indicates that those infants that depleted CD4+ lymphocytes more rapidly were more behaviorally impaired. Many studies in human infants have documented the relationship between lowered CD4+ counts and CNS involvement (Brouwers *et al*, 1994, 1995, Englund *et al*, 1996). However this relationship is not always found (Lindsey *et al*, 2000). In most human studies CD4+ decline is associated with clinical disease progression. In our HIV-2_{287} model, CD4+ decline occurs shortly after infection and prior to clinical disease. The fact that cognitive impairment in this study was associated with lower CD4+ proportions at birth and 2 weeks of age (prior to clinical disease), coupled with data from human studies indicating that increases in CD4+ counts were correlated with improvement in neurocognitive measures during antiretroviral drug therapy (Brouwers *et al*, 1994), gives credence to the argument that lowered CD4+ levels are permissive to CNS involvement. Correlations between CD4+ proportions at birth and 2 weeks were weaker for the Full Hide Well condition. This is not surprising because this cognitive milestone was attained at a later age and more distant in time from the hematological measures. Variability in disease progression could account for this difference.

Dams that gave birth to infected infants had lower proportions and numbers of CD4+ lymphocytes at parturition. This was not unexpected and has been found in human mothers infected with HIV (Abrams *et al*, 1995; Chase *et al*, 2000). However, maternal CD4+ counts at parturition did not predict infant behavioral development. This is not consistent with data presented by Tardieu *et al* (2000), who found that maternal CD4+ counts predicted encephalopathy in their infants. It is possible that our study had insufficient power to detect potential differences, as the sample in the Tardieu study was much larger (426 infants). An alternate explanation is that since HIV-2_{287} causes such rapid and uniform CD4+ lymphocyte depletion there was not enough variability in maternal CD4+ counts to detect differences.

We found no effects of maternal viral dose on maternal CD4+ count at parturition or infant behavioral development. This is not surprising as others have reported that viral dose does not impact disease progression for HIV-2_{287} and other macaque lentiviruses (Hoterman *et al*, 2000; Looney *et al*, 1998). Looney *et al* (1998) found that dose of HIV-2_{287} was unrelated to disease progression and either study dose (10^5 or 10^1) caused a "prompt and profound" decline in CD4+ lymphocytes. The present study also found no effect of infant viral load at birth. Studies in human infants have correlated plasma levels of HIV RNA to behavioral deficits (Gurbindo *et al*, 1999; Pollack *et al*, 1996). However, both of these studies had substantially more subjects. In addition, Gurbindo *et al* (1999) found that other measures (lymphocyte levels) had stronger prognostic value than plasma viral load.

Data from this study indicate that the HIV-2_{287}–infected infant macaque is an excellent animal model of pediatric neuroAIDS. HIV-2_{287}–infected macaque

infants evinced delays in motor and cognitive development that are similar to those seen in HIV-infected infants and children. A considerable strength of the HIV-2_{287} is that it produces marked deficits in behavioral development with little variation. Thus fewer subjects are needed to detect statistically significant differences. HIV-2_{287} has also been shown to produce neuropathology in macaque infants similar to what is seen in HIV-infected human infants (Kinman *et al*, 2004). This nonhuman primate model can be used to explore mechanisms of behavioral pathology associated with lentivirus infection and can also be used to assess the efficacy of therapeutic drug regimens.

Materials and methods

Subjects were 20 infant pigtail macaques (*Macaca nemestrina*), 8 controls and 12 experimental. Dams of experimental infants were inoculated intravenously with HIV-2_{287} in the third trimester of pregnancy ($10TCID_{50}$, $n = 3$; 10^3TCID_{50}, $n = 7$; 10^4TCID_{50}, $n = 2$). All inoculated females became infected (as measured by coculture and polymerase chain reaction [PCR]). Seven infants born to infected dams were vertically infected (as measured by PCR at birth) and five infants were not infected. As described by Ho *et al* (1996, 2001), eight inoculated dams had maternal catheters implanted into their amniotic cavities in the third trimester of pregnancy; in five pregnancies the fetuses were also implanted with carotid artery and jugular vein catheters. Four inoculated dams had no surgical intervention. Dams of two infants received triple drug combination therapy (AZT/DDI [15 mg/kg] and INDV [25 mg/kg, po, tid]) while pregnant. Infants of these dams were HIV-2_{287} negative by both viral RNA and viral coculture on repeated tests. All controls were born to dams that underwent neither surgical interventions nor viral inoculation.

Infants were separated from their mothers at birth and reared in the Washington National Primate Research Center (WaNPRC) BSL 2/3 nursery per the Infant Primate Research Laboratory (IPRL) protocol (Ruppenthal and Sackett, 1992; Worlein *et al*, in press). They were fed formula (Enfamil) according to the IPRL schedule. They were also fed Purina monkey chow, *ad libitum* starting at 14 days of age. Infants were provided with a hanging cloth surrogate to provide contact comfort. They were housed singly in cages that allowed visual, auditory, and olfactory, access to other infants. Infants were socialized with similar-aged peers for 30 min a day, 5 days a week, in a large glass-fronted cage ($36 \times 48 \times 48$ inches, high) that contained ramps, chains, and toys. This method of nursery rearing has been shown to produce infants with normal behavioral repertoires (Worlein and Sackett, 1997).

Object concept methods

This battery of tests measures the infant's ability to recognize that an object still exists after it disappears from direct view. These assessments are based on the Piagetian theory of object permanence and represent cognitive developmental milestones. They have successfully been used to detect developmental delays in nonhuman primate infants (Burbacher *et al,* 1986). The testing apparatus consisted of a white plastic box that had either a small screen in a groove allowing it to be moved from side to side, or a well that could be covered by a removable lid (Figure 4).

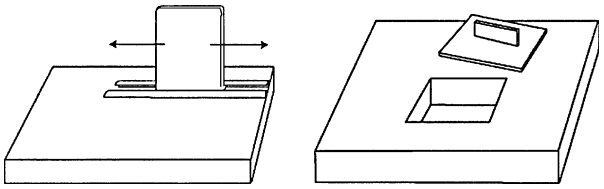

Figure 4 Screen and Well testing apparatuses. A plexiglass block with a moveable opaque screen was used for the Screen Tasks and a plexiglass block with a shallow well that could be covered by an opaque lid was used for the Well Tasks. Objects were placed either in full view (No Hide), partially hidden behind the opaque screen or partially covered by the opaque lid in the well (Partial Hide), or fully hidden behind the opaque screen or totally covered by the opaque lid in the well (Full Hide).

Infants were tested 3 days a week. Testing began after the infants attained the Plain Reach motor milestone (see below). Screen and Well apparatus were alternated on consecutive days of testing. Two persons were involved in the testing procedure. One held the infant swaddled in a cloth diaper and the other tested. Infants were allowed to interact with a stimulus object (a small toy, or at later ages, a piece of fruit), which was then taken from them and placed on the apparatus in one of three types of conditions: No Hide, the object was placed in full view on the apparatus Partial Hide, the object was partially hidden behind the screen or placed in the well and partially covered by the lid; Full Hide, the object was placed totally behind the screen or in the well totally covered by the lid. The infant was given 15 s to make a response. Each session of testing consisted of 15 trials, 5 in each condition (No Hide, Partial Hide, or Full Hide). Criterion is reached for a condition when the animal touched or picked up the object on 8 out of 10 trials over two successive sessions.

Motor development

Two tests of motor development were employed. The first (Plain Reach) assessed the ability of the infant to pick up a small brightly colored toy. Testing began at 14 days of age. Infants were tested 3 days a week. The infant was allowed to interact with a small toy that was then placed on a platform in front of them. Criterion was reached when the infant was successfully able to completely pick up the object on three out of five trials. Fine motor development (Fine Motor Task) was assessed by the ability of the infant to pick up small pieces of fruit between its thumb and index finger in a pincer grasp. Animals were tested three times weekly until they reached criterion of two

successful index finger/thumb pick ups out of five trials.

Hematological assessment
All blood draws were performed under ketamine sedation. Complete blood counts (CBC) and T-cell subset determinations were performed on maternal blood at the time of parturition and on infants at the time of birth and at approximately 2 weeks of age (range: 11 to 16 days). Viral RNA levels were measured at birth by quantitative (QC) reverse transcriptase–polymerase chain reaction (RT-PCR).

Virus
HIV-2_{287} was developed from the culture supernatant of the original isolate of human strain, HIV-2_{EHO}, obtained from Dr. Luc Montagnier at the Pasteur Institute. After four passages in phytohemagglutinin (PHA)-stimulated normal human peripheral blood mononuclear cells (PBMCs), a working stock of HIV-2_{EHO} was prepared and passed twice in *M. nemestrina*. A cell-free virus stock was established from lymph node mononuclear cells collected at euthanasia from one of the second-passage macaques with the clinical manifestations of AIDS (F89287) and cocultured with PHA-stimulated, CD8+-depleted normal macaque PBMCs (McClure *et al*, 2000). This stock, which contained 1×10^5 $TCID_{50}$/ml, was diluted in tissue culture medium for inoculation into macaques.

Coculture methods
Frequency of virus-infected macaque PBMCs was determined by coculture with human lymphoblasts prepared by depleting normal human PBMCs of $CD8^+$ cells and stimulating them for 3 days with 1 μg/ml PHA and 20 IU/ml interleukin (IL)-2 in RPMI containing 10% Nhuserum. Duplicate samples of fivefold serially diluted macaque PBMCs in a fixed volume (2 ml) were added to a constant 1×10^6 lymphoblasts in 24-well tissue culture plates. Plates were incubated for 21 days with cultures being fed fresh medium once every week. The presence of virus in culture supernatants on days 14 and 21 was detected using an HIV-2 p27 antigen capture assay kit (Coulter Corp., Hialeah, FL) according to the manufacturer's instructions. The number of HIV-2–infected macaque PBMCs was estimated from the highest dilution of cells at which detectable HIV-2 antigen was found.

Lymphocyte subsets
Fluorescence-labeled monoclonal antibodies to lymphocyte surface markers were used to quantitate lymphocyte subset populations, including CD2+, CD4+, CD8+, CD20+, in maternal and infant blood using a FACSTAR fluorescence activated cell sorter fitted with an argon-ion laser operating at 488 nm. The data were collected as list-mode files and analyzed on Hewlett-Packard computers using BDIS-CONSORT 30 software.

Viral quantification
The number of copies/ml of viral RNA in plasma were measured using a QC, internally controlled RNA PCR. Total RNA in duplicate plasma samples (200 μl each) was isolated by ultracentrifugation, followed by digestion of the pellet with proteinase-K in the presence of sodium dodecyl sulfate (SDS). The RNA was then extracted from the mixture with phenol:chloroform:iso-amyl alcohol, precipitated with ethanol and pelleted in a microfuge. Contaminating DNA was removed by treatment with DNase. Fourfold serial dilutions of the isolated RNA were made in reverse transcriptase buffer containing 500 copies of a truncated (83-bp deletion) gag RNA internal control that is recognized by the same primers as HIV-2 RNA. Reverse transcriptase reaction was performed in a Perkin Elmer 9600 thermocycler for 15 min at 42°C. Immediately upon cooling, standard PCR reagents (0.15 μM primers and 2.5 units Taq polymerase in PCR buffer containing 3.5 mM magnesium) were added and 45 cycles of heating and cooling were performed as follows: 10 s at 95°C, 30 s at 60°C, and 30 s at 72°C before recycling to 95°C. Amplified RT-PCR products, stained with ethidium bromide, were separated on agarose gels, illuminated at 254 nm, and photographed with a Kodak DC 120 digital camera (Eastman Kodak, Rochester, NY). The 83-bp deletion in the internal control allows discrimination from HIV-2 RNA (254 bp versus 336 bp). HIV-2 RNA levels were calculated from the sample dilution that gave a band intensity equivalent to that of the internal control. Sensitivity of this assay is approximately 400 copies viral RNA/ml of plasma.

Statistical methods
Preliminary analysis indicated that the two infants whose dams received drugs during pregnancy did not differ from the other exposed but not infected infants, therefore their data were collapsed for analysis. Because some dams and infants also underwent catheterization in the prenatal period (for details of surgical procedures see Ho *et al*, 1996), behavioral data were examined to assess possible effects of such interventions. Surgical intervention had little or no effect on behavioral development so data were collapsed for analysis. This yielded three groups: controls (CON), the infants born to uninoculated dams; exposed (EPD), uninfected infants born to inoculated dams; infected (INF), infected infants born to inoculated dams. Data were analyzed by a one-way ANOVA, with group (CON, EPD, and INF) as the independent variable, using SYSTAT statistical software. Post hoc planned comparisons (Fisher LSD) were computed when the overall ANOVA reached statistical significance.

References

Abrams EJ, Matheson PB, Thomas PA, Thea DM, Krasinski K, Lambert G, Shaffer N, Bamji M, Hutson D, Grimm K, *et al* (1995). Neonatal predictors of infection status and early death among 332 infants at risk of HIV-1 infection monitored prospectively from birth. New York City Perinatal HIV Transmission Collaborative Study Group. *Pediatrics* **96:** 451–458.

Angelini L, Zibordi F, Triulzi F, Cinque P, Giudici B, Pinzani R, Plebani A (2000). Age-dependent neurologic manifestations of HIV infection in childhood. *Neurol Sci* **21:** 135–142.

Aylward EH, Butz AM, Hutton N, Joyner ML, Vogelhut JW (1992). Cognitive and motor development in infants at risk for human immunodeficiency virus. *Am J Dis Child* **146:** 218–222.

Belman AL (1990). AIDS and pediatric neurology. *Neurol Clin* **8:** 571–603.

Belman AL (1992). Acquired immunodeficiency syndrome and the child's central nervous system. *Pediatr Clin N Am* **39:** 691–714.

Belman AL (2002). HIV-1 infection and AIDS. *Neurol Clin* **20:** 983–1011.

Belman AL, Diamond G, Dickson D, Horoupian D, Llena J, Lantos G, Rubinstein A (1988). Pediatric acquired immunodeficiency syndrome: neurologic syndromes. *Am J Dis Child* **142:** 29–35.

Belman AL, Muenz LR, Marcus JC, Goedert JJ, Landesman S, Rubinstein A, Goodwin S, Durako S, Willoughby A (1996). Neurologic status of human immunodeficiency virus 1-infected infants and their controls: a prospective study from birth to 2 years. *Pediatrics* **98:** 1109–1118.

Boivin MJ, Davies AG, Mokili JKL, Green SDR, Giordani B, Cutting WAM (1995). A preliminary evaluation of cognitive and motor effects of pediatric HIV infection in Zairian children. *Health Psychol* **14:** 13–21.

Brouwers P, DeCarli C, Tudor-Williams G, Civitello L, Moss H, Pizzo P (1994). Interrelations among patterns of change in neurocognitive, CT brain imaging and CD4 measures associated with anti-retroviral therapy in children with symptomatic HIV infection. *Adv Neuroimmuno* **4:** 223–231.

Brouwers P, Tudor-Williams G, DeCarli C, Moss HA, Wolters PL, Civitello LA, and Pizzo PA (1995). Relation between stage of disease and neurobehavioral measures in children with symptomatic HIV disease. *AIDS* **9:** 713–720.

Bruck I, Tahan TT, Rodrigues da Cruz C, Martins LTF, Antoniuk SA, Rodrigues M, Mara de Souza S, Regina de Bruyn L (2001). Developmental milestones of vertically HIV infected and seroreverters Children: follow up of 83 children. *Arq Neuropsiquiatr* **59:** 691–695.

Burbacher TM, Grant KS, Mottet NK (1986). Retarded object permanence development in methylmercury exposed *macaca-fascicularis* infants. *Dev Psychol* **22:** 771–776.

Chase C, Vibbert M, Pelton SI, Coulter DL, Cabral H (1995). Early neurodevelopmental growth in children with vertically transmitted human immunodeficiency virus infection. *Arch Pediatr Adolesc Med* **149:** 850–855.

Chase C, Ware J, Hittelman J, Blasini I, Smith R, Llorente A, Anisfeld E, Diaz C, Fowler MG, Moye J, Kaligh LI (2000). Early cognitive and motor development among infants born to women infected with human immunodeficiency virus. *Pediatrics* **106:** e25.

Chougnet C, Kovacs A, Baker R, Mueller BU, Luban NL C, Liewehr DJ, Steinberg SM, Thomas EK, Shearer GM (2000). Influence of human immunodeficiency virus-infected maternal environment on development of infant interleukin-12 production. *J Infect Dis* **181:** 1590–1597.

Clerici M, Saresella M, Colombo F, Fossati S, Sala N, Bricalli D, Villa ML, Ferrante P, Dally L, Vigano A (2000). T-lymphocyte maturation abnormalities in uninfected newborns and children with vertical exposure to HIV. *Blood* **96:** 3866–3871.

Cooper ER, Hanson C, Diaz C, Mendez H, Abboud R, Nugent R, Pitt J, Rich K, Rodrigues EM, Smeriglio V (1998). Encephalopathy and progression of human immunodeficiency virus disease in a cohort of children with perinatally acquired human immunodeficiency virus infection. *J Pediatr* **132:** 808–812.

Coplan J, Contello KA, Cunningham CK, Weiner LB, Dye TD, Roberge L, Wojtowycz MA, Kirkwood K (1998). Early language development in children exposed to or infected with human immunodeficiency virus. *Pediatr Electronic* **102:** e8.

Coscia JM, Christensen BK, Henry RR, Wallston K, Radcliffe J, Rutstein R (2001). Effects of home environment, socioeconomic status, and health status on cognitive functioning in children with HIV-1 infection. *J Pediatr Psychiatry* **26:** 321–329.

DeMaria MA, Casto M, O'Connell M, Johnson RP, Rosenzweig M (2000). Characterization of lymphocyte subsets in rhesus macaques during the first year of life. *Eur J Haematol* **65:** 245–257.

Diamond GW (1989). Developmental problems in children with HIV infection. *Mental Retard* **27:** 213–217.

Diamond GW, Kaufman J, Belman AL, Cohen L, Cohen HJ, Rubinstein A (1987). Characterization of cognitive functioning in a subgroup of children with congenital HIV infection. *Arch Clin Neuropsychol* **2:** 245–256.

Diamond GW, Gurdin P, Wiznia AA, Bleman AL, Rubenstein A, Cohen HJ (1990). Effects of congenital HIV infection on neurodevelopmental status of babies in foster care. *Dev Med Child Neurol* **32:** 999–1005.

Drotar D, Olness K, Wiznitzer M, Guay L, Marum L, Svilar G, Hom D, Fagan JF, Ndugwa C, Kiziri-Mayengo R (1997). Neurodevelopmental outcomes of Ugandan infants with human immunodeficiency virus type 1 infection. *Pediatrics* **100:** E5.

Drotar D, Olness K, Wiznitzer M, Schatschneider C, Marum L, Guay L, Fagan J, Hom D, Svilar G, Ndugwa C, Mayengo RK (1999). Neurodevelopmental outcomes of Ugandan infants with HIV infection: an application of growth curve analysis. *Health Psychol* **18:** 114–121.

Eiden LE, Rausch DM, da Cunha A, Murray EA, Heyes M, Sharer L, Nohr D, Weihe E (1993). Aids and the central nervous system. *Ann N Y Acad Sci* **693:** 229–244.

Englund JA, Baker CJ, Raskino C, McKinney RE, Lifschitz MH, Petrie B, Fowler MG, Connor JD, Mendez H, O'Donnell K, Wara DW, The Aids Clinical Trials Group Protocol 152 Study Team (1996). Clinical and laboratory characteristics of a large cohort of symptomatic, human immunodeficiency virus-infected infants and children. *Pediatr Infect Dis J* **15:** 1025–1036.

Epstein LG (1988). HIV infection in the newborn and child: specific effects on the nervous system. In: *AIDS in children, adolescents, and heterosexual adults*. Schinazi RF, and Nahmias AJ (eds). New York: Elsevier Science, pp 241–244.

Epstein LG, Sharer LR, Joshi VV, Fojas MR, Koenigsberger MR, Oleske JW (1984). Progressive encephalopathy in children with acquired immunodeficiency syndrome. *Ann Neurol* **17:** 488–496.

Epstein LG, Sharer LR, Oleske JM, Connor EM, Goudsmit J, Bagdon L, Robert-Guroff M, Koenigsberger MR (1986). Neurologic manifestations of human immunodeficiency virus infection in children. *Pediatr* **78:** 678–687.

Esposito S, Musetti L, Musetti MC, Tornaghi R, Corbella S, Massironi E, Marchisio P, Guareschi A, Principi N (1999). Behavioral and psychological disorders in uninfected children aged 6 to 11 years born to human immunoedeficiency virus-seropositive mothers. *J Dev Behav Pediatr* **20:** 411–417.

Fox HS, Buchmeier MJ, Lane TE, Stenglein S, Nelson J, Prospero-Garcia O, Henriksen SJ, Gold LH, Bloom FE (1996). Infection of rhesus macaques with neuropathogenic SIV induces cognitive and electrophysiologic abnormalities: viral and host factors lead to CNS dysfunction. *J NeuroVirol* **2:** 13.

Fox HS, Gold LH, Henriksen SJ, Bloom FE (1997). Simian immunodeficiency virus: a model for neuroAIDS. *Neurobiol Dis* **4:** 265–274.

Gesner M, Papaevangelou V, Kim M, Chen SH, Moore T, Krasinski K, Borkowsky W (1994). Alteration in the proportion of CD4 T lymphocytes in a subgroup of human immunodeficiency-virus-exposed-uninfected children. *Pediatr* **93:** 624–630.

Gold LH, Fox HS, Henriksen SJ, Buchmeier MJ, Weed MR, Taffe MA, Huitron-Resendiz S, Horn TF, Bloom FE (1998). Longitudinal analysis of behavioral, neurophysiological, viral and immunological effects of SIV infection in rhesus monkeys. *J Med Primatol* **27:** 104–112.

Gurbindo D, Resino S, Sanchez-Ramon S, Leon JA, Munoz-Fernandez MA (1999). Correlation of viral load and CD8 T-lymphocytes with development of neurological manifestations in vertically HIV-1-infected infants. A prospective longitudinal study. *Neuropediatrics* **30:** 197–204.

Herz AM, Robertson MN, Lynch JB, Schmidt A, Rabin M, Sherbert C, Agy MB, Anderson D, Hu SL, Greenberg PD, Morton WR (2002). Viral dynamics of early HIV infection in neonatal macaques after oral exposure to HIV-2287: an animal model with implications for maternal-neonatal HIV transmission. *J Med Primatol* **31:** 29–39.

Ho RJY, Agy MB, Morton WR, Scheibel M, McClure J, Watson A, Hu SL, Nosbisch C, Dorofeeva N, Unadkat JD (1996). Development of a chronically catheterized maternal-fetal macaque model to study in utero mother-to-fetus HIV transmission: a preliminary report. *J Med Primatol* **25:** 218–224.

Ho RJ, Larsen K, Kinman L, Sherbert C, Wang XY, Finn E, Nosbisch C, Schmidt A, Anderson D, Hu SL, Agy M, Ochs H, Morton WR, Unadkat JD (2001). Characterization of a maternal-fetal HIV transmission model using pregnant macaques infected with HIV-2 (287). *J Med Primatol* **30:** 131–140.

Holterman L, Niphuis H, Koornstra W, Dubbes R, ten Haaft P, Heeney JL (2000). The rate of progression to AIDS is independent of virus dose in simian immunodeficiency virus-infected macaques. *J Gen Virol* **81:** 1719–1726.

Horn TF, Huitron-Resendiz S, Weed MR, Henriksen SJ (1998). Early physiological abnormalities after simian immunodeficiency virus infection. *Proc Nat Acad Sci U S A* **95:** 15072–15077.

Kinman LM, Worlein JM, Leigh J, Bielefeldt-Ohmann H, Anderson DM, Hu S-L, Morton WR, Anderson BD, Ho RJY (2004). HIV in central nervous system and behavioral development: An HIV-2_{287} macaque model of AIDS. *AIDS* **18:** 1363–1370.

Knight WG, Mellins CA, Levenson RL Jr., Arpadi SM, Kairam R (2000). Brief report: effects of pediatric HIV infection on mental and psychomotor development. *J Pediatr Psychol* **25:** 583–587.

Kuhn L, Coutsoudis A, Moodley D, Mngqundaniso N, Trabattoni D, Shearer GM, Clerici M, Coovadia HM (2001). Interferon-gamma and interleukin-10 production among HIV-1-infected and uninfected infants of HIV-1-infected mothers. *Pediatr Res* **50:** 412–416.

Kuhn L, Meddows-Taylor S, Gray G, Tiemessen C (2002). Human immunodeficiency virus (HIV)-specific cellular immune responses in newborns exposed to HIV in utero. *CID* **34:** 267–276.

Lifschitz M, Hanson C, Wilson G, and Shearer WT (1989). Behavioral changes in children with human immunodeficiency virus (HIV) infection. International Conference on AIDS: Abstracts, 5, 316.

Lindsey JC, Hughes MD, McKinney RE, Cowles MK, Englund JA, Baker CJ, Burchett SK, Kline MW, Kovacs A, Moye J (2000). Treatment-mediated changes in human immunodeficiency virus (HIV) type 1 RNA and CD4 cell counts as predictors of weight growth failure, cognitive decline, and survival in HIV-infected children. *J Infect Dis* **182:** 1385–1393.

Lobato MN, Caldwell MB, Ng P, Oxtoby MJ, Pediatric Spectrum of Disease Clinical Consortium (1995). Encephalopathy in children with perinatally acquired human immunodeficiency virus infection. *J Pediatr* **126:** 710–715.

Looney DJ, McClure J, Kent SJ, Radaelli A, Kraus G, Schmidt A, Steffy K, Greenberg P, Hu SL, Morton WR, Wong-Stall F (1998). A minimally replicative HIV-2 live-virus vaccine protects *M. nemestrina* from disease after HIV-2(287) challenge. *Virology* **242:** 150–160.

Lucas SB, Hounnou A, Peacock C, Beaumel A, Djomand G, N'Gbichi JM, Yeboue K, Honde M, Diomande M, Giordano C, Doorly R, Brattegaard K, Kestens L, Smithwick R, Kadio A, Ezani N, Yapi A, De Cock KM (1993). The mortality and pathology of HIV infection in a West African city. *AIDS* **7:** 1569–1579.

Macmillan C, Magder LS, Brouwers P, Chase C, Hittelman J, Lasky T, Malee K, Mellins CA, Velez-Borras (2001). Head growth and neurodevelopment of infants born to HIV-1-infected drug-using women. *J Neurol* **57:** 1402–1411.

Marcario JK, Raymond LAM, McKiernan BJ, Foresman LL, Joag SV, Raghavan R, Narayan O, Cheney PD (1999). Motor skill impairment in SIV-infected rhesus macaques with rapidly and slowly progressing disease. *J Med Primatol* **28:** 105–117.

McClure J, Schmidt AM, Rey-Cuille M-A, Bannick J, Misher L, Tsai CC, Anderson DM, Morton WR, Hu SL (2000). Derivation and characterization of a highly pathogenic isolate of human immunodeficiency virus type 2 that

causes rapid CD4+ cell depletion in *Macaca nemestrina*. *J Med Primatol* **29:** 114–126.

Mellins CA, Levenson RL Jr, Zawadzki R, Kairam R, Weston M (1994). Effects of pediatric HIV infection and prenatal drug exposure on mental and psychomotor development. *J Pediatr Psychol* **19:** 617–628.

Mintz M (1994). Clinical comparison of adult and pediatric NeuroAIDS. *Adv Neuroimmunol* **4:** 207–221.

Mintz M (1998). Clinical features of HIV infection in children. In: *The neurology of AIDS*. Gendelman HE, Liption SA, Epstein L, Swindells S (eds). New York: Chapman & Hall, pp 385–407.

Moss HA, Brouwers PL, Wolters PL, Wiener L, Hersh S, Pizzo PA, (1994). The development of a Q-sort behavioral rating procedure for pediatric HIV patients. *J Pediatr Psychol* **19:** 27–46.

Moss HA, Wolters PL, Brouwers P, Hendricks ML, Pizzo PA (1996). Impairment of expressive behavior in pediatric HIV-infected patients with evidence of CNS disease. *J Pediatr Psychol* **21:** 379–400.

Moss HA, Wolters PL, Eddy J, Weiner L, Pizzo P, Brouwers P (1989). The effects of encephalopathy and AZT treatment on the social and emotional behavior in pediatric AIDS. V International Conference on AIDS: Abstracts, 5, 328.

Moss HA, Wolters PL, El-Amin D, Butler K, Brouwers P, Pizzo P (1990). The use of videotaped behavior samples of pediatric AIDS patients to evaluate psychosocial changes associated with encephalopathy before and after treatment. VI International Conference on AIDS: Abstracts, 6, 131.

Murray EA, Rausch DM, Lendvay J, Sharer LR, Eiden LE (1992). Cognitive and motor impairments associated with SIV infection in Rhesus monkeys. *Science* **255:** 1246–1249.

Nielsen SD, Jeppesen DL, Kolte L, Clark DR, Sorensen TU, Dreves AM, Ersboll AK, Ryder LP, Valerius NH, Nielsen JO (2001). Impaired progenitor cell function in HIV-negative infants of HIV-positive mothers results in decreased thymic output and low CD4 counts. *Blood* **98:** 398–404.

Nozyce M, Hittelman J, Muenz L, Duraki SJ, Fischer ML, Willoughby A (1994). Effect of perinatally acquired human immunodeficiency virus infection on neurodevelopment in children during the first two years of life. *Pediatr* **94:** 883–891.

Pearson DA, McGrath NM, Nozyce M, Nichols SL, Raskino C, Brouwers P, Lifschitz MC, Baker CJ, Englund JA (2000). Predicting HIV disease progression in children using measures of neuropsychological and neurological functioning. *Pediatrics* **106:** e76.

Pollack H, Kuchuk A, Cowan L, Hacimamutoglu S, Glasberg H, David R, Krasinski K, Borkowsky W, Oberfield S (1996). Neurodevelopment, growth, and viral load in HIV-infected infants. *Brain Behav Immun* **10:** 298–312.

Pratt RD, Nichols S, McKinney N, Kwok S, Danker WM, Spector SA (1996). Virologic markers of human immunodeficiency virus type 1 in cerebrospinal fluid of infected children. *J Infect Dis* **174:** 288–293.

Price RW, Brew B, Sidtis JJ, Rosenblum M, Scheck A, Cleary P (1988). The brain in AIDS: central nervous system HIV-1 infection and AIDS dementia complex. *Science* **239:** 586–592.

Prospero-Garcia O, Gold LH, Fox HS, Polis I, Koob GF, Bloom FE, Henriksen SJ (1996). Microglia-passaged simian immunodeficiency virus induces neurophysiological abnormalities in monkeys. *Proc Nat Acad Sci U S A* **93:** 14158–14163.

Rausch DM, Heyes MP, Eiden LE (1994a). Effects of chronic zidovudine administration on CNS function and virus burden after perinatal SIV infection in rhesus monkeys. *Adv Neuroimmunol* **4:** 233–237.

Rausch DM, Heyes MP, Murray EA, Eiden LE (1995). Zidovudine treatment prolongs survival and decreases virus load in the central nervous system of Rhesus macaques infected perinatally with simian immunodeficiency virus. *J Infect Dis* **172:** 59–69.

Rausch DM, Heyes MP, Murray EA, Lendvay J, Sharer LR, Ward JM, Rehm S, Nohr D, Weibe E, Eiden LE (1994b). Cytopathologic and neurochemical correlates of progression to motor/cognitive impairment in SIV-infected rhesus monkeys. *J Neuropathol Exp Neurol* **53:** 165–175.

Raymond LAM, Wallace D, Marcario JK, Raghavan R, Narayan O, Foresman LL, Berman NEJ, Cheney PD (1999). Motor evoked potentials in a rhesus macaque model of neuro-AIDS. *J NeuroVirol* **5:** 217–231.

Ruppenthal GC, Sackett GP (1992). Research protocol and technician's manual: a guide to the care, feeding and evaluation of infant monkeys. Seattle, WA: The Infant Primate Research Laboratory.

Schwarcz SK, Rutherford GW (1989). Acquired immunodeficiency syndrome in infants, children and adolescents. *J Drug Issues* **19:** 75–92.

Scott GB, Hutto C, Makuch RW, Mastrucci MT, O'Conner T, Mitchell CD, Trapido EJ, Parks WP (1989). Survival in children with perinatally acquired human immunodeficiency virus type I infection. *N Engl J Med* **321:** 1791–1796.

Sharer LR, Epstein LG, Cho ES, Joshi VV, Meyenhofer MF, Rankin LF, Petito CK (1986). Pathologic features of AIDS encephalopathy in children: evidence for LAV/HTLV-III infection of the brain. *Human Pathol* **17:**271–284.

Sharer LR, Saito Y, Blumberg BM (1997). Neuropathology of Human Immunodeficiency Virus-1 infection of the brain. In: *Aids and the nervous system*, 2nd ed. Berger JR and Levy RM (eds). Philadelphia: Lippincott-Raven Publishers, 461–479.

Smith R, Malee K, Charurat M, Magder L, Mellins C, MacMillan C, Hittleman J, Lasky T, Llorente A, Moye J (2001). Timing of perinatal human immunodeficiency virus type 1 infection and rate of neurodevelopment. *Pediatr Infect Dis J* **19:** 862–871.

Tardieu M, Mayaux MJ, Seibel N, Funck-Brentano I, Straub E, Teglas JP, Blanche S (1995). Cognitive assessment of school-age children infected with maternally transmitted human immunodeficiency virus type 1. *J Pediatr* **126:** 375–379.

Tardieu M, Chenadec JL, Persoz A, Meyer L, Blanche S, Mayaux MJ (2000). Hiv-1-related encephalopathy in infants compared with children and adults. *Neurol* **54:** 1089–1095.

Terao K, Rose LM, Sackett GP, Clark EA (1988). Development of lymphocyte subsets in pigtailed macaques. *Human Immunol* **21:** 33–48.

Tovo PA, de Martino M, Gabiano C, Cappello N, D'Elia R, Loy A, Plebani A, Zuccotti GV, Dallacasa P, Ferraris G, Caselli D, Fundaro' C, D'Argenio P, Galli L, Principi N, Stegagno M, Ruga E, Palomba E, The Italian Register

for HIV Infection in Children (1992). Prognostic factors and survival in children with perinatal HIV-1 infection. *Lancet* **339:** 1249–1253.

Watson A, McClure J, Ranchalix J, Scheibel M, Schmidt A, Kennedy B, Morton WR, Haigwood NL, Hu SL (1997). Early postinfection antiviral treatment reduces viral load and prevents CD4+ cell decline in HIV type 2-infected macaques. *AIDS Res Hum Retroviruses* **13:** 1375–1381.

Westmoreland SV, Williams KC, Simon MA, Bahn ME, Rullkoetter AE, Elliott MW, deBakker CD, Knight HL, Lackner AA (1999). Neuropathogenesis of simian immunodeficiency virus in neonatal rhesus macaques. *Am J Pathol* **155:** 1217–1228.

Wilkins JW, Robertson KR, van der Horst C, Robertson WT, Fryer JG, Hall CD (1990). The importance of confounding factors in the evaluation of neuropsychological changes in patients infected with human immunodeficiency virus. *J Acquir Immune Defic Synd* **3:** 938–942.

Wolters PL, Brouwers P, Moss HA, Pizzo PA (1994). Adaptive behavior of children with symptomatic HIV infection before and after Zidovudine therapy. *J Pediatr Psychol* **19:** 47–61.

Worlein JM, Ha JC, Harris C, Leigh J, Stratton K, Ho JR (in press). Special challenges of rearing infant macaques infected with lentivirus (SIV, HIV, SHIV). In: *Developments in primatology: progress and prospects*. Sackett GP and Ruppenthal G (eds).

Worlein JM, Sackett GP (1997). Social development in nursery-reared infant pigtailed macaques (Macaca nemestrina). *Am J Primatol* **41:** 23–35.