American Journal of Pathology, Vol. 149, No. 4, October 1996

Short Communication

Localization of Simian Immunodeficiency Virus Nucleic Acid and Antigen in Brains of Fetal Macaques Inoculated *in Utero*

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Neurological dysfunction has been shown to be associated with human immunodeficiency virus (HIV) infection. The incidence of these abnormalities is greater in HIV-infected children when compared with adults, and the patterns of neurological disease are also known to differ from those observed in the adult population. The reasons for these differences are unclear but are most likely related to the immaturity of the host's immune and central nervous systems at the time of infection. This is thought to be particularly true for infants infected with HIV prenatally. To examine these questions, the brains of fetal rhesus macaques that were infected with SIVmac251 at various time points* in utero *were examined. Direct fetal inoculations were performed on gestational day (GD) 65 (n = 8; early second trimester), GD 110 (n = 4; early third trimester) and GD 130 (n = 2; mid third trimester), with harvest of fetal tissues on GD 80, 100, 130, or 145. Eleven sham controls were included with harvest at correlative time points. Specimens were examined by routine histology, immunohistochemistry, and* in situ *hybridization to localize viral antigens and SIV nucleic acid. Histologically, scattered glial nodules, spongiosis, and mineralization were found in the basal ganglia and deep white matter in 4 of the 14 fetuses (3 inoculated on GD 65 and one on GD 110). These fetuses and those without histological lesions had viral nucleic acid and SIV antigen in the stroma of the choroid plexus, meninges, and external granular layer of the cerebellum and in columns of cells in the cortical plate. In contrast to juvenile and adult macaques, very few SIV-positive perivascular mononuclear cells were present. These findings suggest that SIV has a different distribution in the brain of fetal macaques after direct infection when compared with adult or juvenile animals. Furthermore, the results of these studies suggest that differences in neurological disease between pediatric and adult patients with acquired immune deficiency syndrome are most likely related to the time of infection. (Am J Pathol 1996, 149:1097–1104)

Pediatric human immunodeficiency virus (HIV) infection is frequently associated with neurological symptoms such as a progressive or static loss of previously acquired developmental milestones.[1] In addition, brain growth has been shown to be impaired, with subsequent atrophy of the parenchyma often found at autopsy.[1] In contrast to the neurological disease in adults, disease in infants is characterized by a lower incidence of opportunistic infections, peripheral neuropathies, and vacuolar myelopathy and by vessel-associated mineralization primarily in the basal ganglia.[1–3] The presence of

Supported by Public Health Service grant NS30769 from the National Institute of Neurologic Disorders and Stroke and grants RR00169 and RR00168 from the National Center for Research Resources.

Accepted for publication May 22, 1996.

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multinucleated giant cells is characteristic of HIV encephalitis in both adult and pediatric patients, although it seems to be more frequent in adults than young children.[4,5] Although extensive studies have been performed on the brains of adult patients with acquired immune deficiency syndrome (AIDS), relatively few studies have examined the distribution of HIV in young patients. These studies have identified lentiviral particles, HIV antigens, and nucleic acid in the brain and spinal cord of the human fetus and neonate, primarily in macrophages, microglia, and multinucleated giant cells similar to the distribution of HIV in adults.[2,3,6–8] Unique to pediatric AIDS patients is the possibility of restricted infection of astrocytes suggested by two recent reports.[9,10]

The reasons for the differences in clinical course, neuropathology, and viral distribution in the central nervous system (CNS) when comparing adult and pediatric AIDS patients are obscure. A likely explanation is the timing of infection relative to the immaturity of the host's immune system and development of the CNS. This may be particularly relevant for the fetal CNS, which has varying levels of mitotic activity throughout gestation, possibly providing a better substrate for viral replication than during postnatal life. More information about the effects of viral infection at different developmental stages of the CNS and about the distribution of virus in the developing CNS is needed to address these questions and develop successful intervention strategies. To do so, however, requires a suitable animal model.

Attempts to develop an animal model have utilized the simian immunodeficiency virus (SIV), which is an immunosuppressive lentivirus closely related to HIV.[11,12] To focus on issues related to fetal pathogenesis, a direct *in utero* SIV inoculation monkey model has been established and previously described.[13,14] Using tissues from these studies, we report here the localization of SIV in the developing fetal brain as it relates to the length and timing of infection.

Materials and Methods

Animals

To examine the effects of *in utero* SIV infection in the developing fetal brain, 25 fetal rhesus macaques (14 infected and 11 noninfected) were examined. Methods for housing, direct fetal SIV inoculation (intraperitoneal via ultrasound guidance), *in utero* sonographic monitoring, and hysterotomy have been previously described.[13,14] Briefly, the inoculum consisted of 10^3 tissue culture infectious doses ($TCID_{50}$) of cell-free SIVmac251. This dose of SIVmac251 has been shown to routinely induce infection and AIDS by either the intravenous or mucosal routes in juvenile and adult macaques.[15] Fetuses were directly inoculated on gestational day (GD) 65 (n = 8; early second trimester), GD 110 (n = 4; early third trimester), and GD 130 (n = 2; mid third trimester). Gestation in rhesus macaques is 165 ± 10 days. Age-matched, mock-inoculated control fetuses (n = 11) received saline only under the same conditions. A variety of abnormalities were seen in 13 of the 14 fetuses infected with SIV including intrauterine growth restriction, oligohydramnios, generalized lymphadenopathy, and thymic atrophy. Detailed results of these studies and discussion of necropsy findings have been reported previously[13,14] and are presented in Table 1.

Tissue Collection

At hysterotomy, blood was collected for viral culture and the fetuses were euthanized, as required, by an overdose of sodium pentobarbital. Multiple specimens were collected,[13] and sections of cerebrum, including parietal cortex and basal ganglia, cerebellum and choroid plexus, were immediately snap-frozen in OCT compound (Miles Scientific, Elkhart, IN) by immersion in liquid-nitrogen-cooled freon and stored at −70°C. An adjacent block of tissue was fixed in 10% neutral buffered formalin. Cryostat sections, 6 μm thick, were examined by immunohistochemistry for viral antigen. Formalin-fixed tissues were embedded in paraffin and sectioned at 6 μm for routine histopathological examination and *in situ* hybridization for SIV nucleic acids.

Virus Isolation

Infectious virus was isolated from peripheral blood of each fetus by co-cultivation of 5×10^6 CEMX174 cells with at least 1×10^6 peripheral blood mononuclear cells as described previously.[13,14,16] Cultures were considered positive when culture supernatants yielded 10 ng/ml or more of SIV p27 at two consecutive time points. All cultures were maintained for 8 weeks and tested for SIV p27 by enzyme immunoassay before being scored as virus negative.

Localization of SIV-Infected Cells by in Situ *Hybridization and Immunohistochemistry*

Viral localization was performed by immunohistochemistry for viral antigens and *in situ* hybridiza-

Table 1. *Clinical and Pathological Findings in SIV-Infected Fetuses Grouped by Gestational Day of Inoculation*[1,3]

GD of inoculation	GD of necropsy	Animal	Clinical and pathological observations	Neuropathology
65	80	923-0197	NSL	NSL
		923-0593	Generalized lymphadenopathy	NSL
	100	923-0134	Generalized lymphadenopathy, thymic atrophy, IUGR	Scattered glial nodules in the cerebrum
		923-0206	IUGR, mild oligohydramnios	NSL
		923-0273	Generalized lymphadenopathy, thymic atrophy, severe IUGR	NSL
	130	923-0136	IUGR	Spongiosis, diffuse, mild, in the cerebrum
		923-0215	Generalized lymphadenopathy, thymic atrophy, IUGR	NSL
	145	923-0228	Generalized lymphadenopathy, emaciation, hemorrhage in thymus, lymph nodes, and liver	Mineralization, multifocal with gliosis and spongiosis, in the cerebrum
110	130	923-0223	Mild IUGR, emaciation, oligohydramnios	NSL
		923-0246	Emaciation	NSL
	145	923-0204	Emaciation, thymic atrophy	Scattered glial nodules and multifocal mineralization in the cerebrum
		923-0663	Fetal death at GD 138	NSL
130	145	923-0205	Thymic atrophy	NSL
		923-0625	Emaciation	NSL

IUGR, intrauterine growth restriction; NSL, no significant lesions.

tion for viral DNA and RNA. Two *in situ* hybridization protocols were used. The first technique used a DNA probe labeled with digoxigenin by random priming and the second used radiolabeled RNA probes. The DNA probe used was a combination of two plasmids: 1) a subclone of p239SpE3′, which contains *tat*, *rev*, *env*, *nef*, and a small part of the 3′ long terminal repeat in pBS^-, and 2) p239SpSp5′, which contains *gag*, *pol*, *vif*, *vpx*, *vpr*, and the 5′ long terminal repeat in pBS^+.[17] This combination provides essentially the entire SIVmac239 genome. The probe was labeled with digoxigenin-dUTP by random priming (Boehringer Mannheim, Indianapolis, IN). Probe size and labeling were determined by electrophoresis, blotting, and immunostaining of the DNA with anti-digoxigenin antibodies. Hybridization and detection procedures have been previously described[18,19] and were performed under denaturing conditions to detect both viral DNA and RNA. As a negative control, sections were hybridized with plasmid pUC19, which had been labeled with digoxigenin at the same time as the probe. Tissues from age-matched, mock-inoculated fetuses served as an additional control.

To confirm the results obtained from this technique, selected paraffin blocks of brain were sent to Dr. Cecil H. Fox (Molecular Histology, Gaithersburg, MD) for *in situ* hybridization for SIV RNA as previously described,[20] using radiolabeled RNA probes synthesized from five DNA templates, covering 90% of the SIV genome, subcloned into pGEM4. Controls consisted of matched tissues from mock-inoculated fetuses and hybridizing sections with the sense probe.

Sections were examined microscopically, and different brain regions were scored semiquantitatively on a scale of 0 to 3+ as follows (Table 2): 0, no positive cells; 1+, 1 to 4 positive cells per region, 2+, 5 to 10 positive cells per region, and 3+, >10 positive cells per region.

To localize viral antigen, snap-frozen tissues were used in immunohistochemical procedures as previously described.[19,21] Briefly, tissue sections were fixed in 2% paraformaldehyde for 10 minutes at 4°C and immunostained using an avidin-biotin-horseradish peroxidase complex (ABC) technique with diaminobenzidine as the chromogen. The primary antibodies used were R1C7 (A. A. Minassian and M. Popovic, National Institutes of Health, Bethesda, MD) and Senv71.1 (C. Colignon and C. Thiriart, Smith-Kline Beacham, Belgium), which recognize SIV p27 and gp120, respectively. Negative controls included serial sections processed identically using equivalent concentrations of irrelevant primary antibodies of the same isotype and matched tissues from mock-inoculated fetuses.

Table 2. *Localization of SIV Nucleic Acid in the CNS by* in Situ *Hybridization*

GD of inoculation	GD of necropsy	Animal	Cortical white matter	Cortical plate	Basal ganglia	Meninges	Choroid plexus	Cerebellum
65	80	923-0197	+	0	0	+	0	NE
		923-0593	+	0	0	+	0	NE
	100	923-0134	0	0	0	+	+	0
		923-0206	0	+	0	0	+	0
		923-0273	+	0	0	+	0	+ (EGL)
	130	923-0136	+ (PV)	0	0	0	0	NE
		923-0215	+	++	+	++	+	NE
	145	923-0228	0	+	0	+	+	NE
110	130	923-0223	+	+	++	0	+	NE
		923-0246	+	+	+	0	+	+++ (EGL)
	145	923-0204	+	0	0	0	0	0
		923-0663	0	+	0	0	+	NE
130	145	923-0205	+ (PV)	+	0	++	0	++ (EGL)
		923-0625	+	++	+	+	0	0

Sections were examined microscopically, and different brain regions were subjectively quantified on a scale of 0 to 3+ as follows: 0, no positive cells; 1+, 1 to 4 positive cells per region; 2+, 5 to 10 positive cells per region; 3+, >10 positive cells per region. EGL, external granular layer of the cerebellum; PV, perivascular; NE, not examined.

Results

Neuropathology

Four of the fourteen fetal rhesus macaques inoculated *in utero* had CNS lesions (Table 1). The lesions were mild and consisted of scattered glial nodules, spongiosis, and multifocal mineralization in the basal ganglia and deep white matter (Figure 1). None of the animals had multinucleated giant cells characteristic of SIV encephalitis[21] or perivascular cuffs, which are commonly seen early in infection of juvenile macaques.[22–24] Three of these four macaques with CNS lesions had been inoculated at GD 65 and one at GD 110. All four of these animals had been infected at least 35 days at the time of necropsy. No correlation between neuropathology and GD of inoculation or length of infection was apparent. Neuropathology, however, was only observed in the SIV-inoculated macaques. None of the age-matched, mock-inoculated fetuses had any recognized lesions in the CNS.

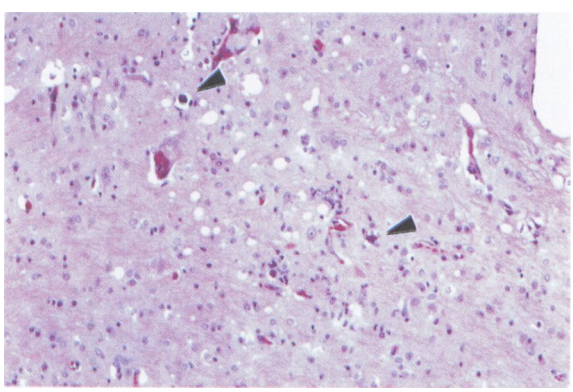

Figure 1. *Mineralization* (arrowheads), *spongiosis, and gliosis in the deep white matter of the cerebral cortex in fetus 923-0228 collected at GD 145. The animal had been inoculated on GD 65. H&E; magnification, ×90.*

Viral Localization

All 14 SIV-inoculated fetuses had virus in the CNS regardless of GD of inoculation, time after infection, or presence of lesions in the CNS. Virus-positive cells were present in the meninges, cortical white matter, basal ganglia, stroma of the choroid plexus, external granular layer of the cerebellum, and the cortical plate (Table 2 and Figure 2). The cortical white matter was the most common location in which to find SIV-positive cells (10 of 14 animals) followed closely by the meninges (8 of 14), cortical plate (8 of 14), and choroid plexus (7 of 14) and distantly by the basal ganglia (4 of 14). The number of infected cells detected was low with no more than 30 positive cells seen in the brain of any animal. No significant differences were noted in the number or distribution of virus-positive cells between any of the groups, although fetuses harvested on GD 130 or later tended to have more positive cells regardless of GD of inoculation.

Despite the apparent low level of infection, similar numbers of infected cells were detected using either nonradioactive *in situ* hybridization performed under denaturing conditions, which detects both viral DNA and RNA, or by *in situ* hybridization using radiolabeled riboprobes, which detect only viral RNA. Fewer positive cells were detected by immunohistochemistry for viral antigen, although the distribution within the CNS was identical to that seen by *in situ* hybridization (Figure 2).

Cells positive for viral antigen or nucleic acid did not appear to be associated with histological lesions.

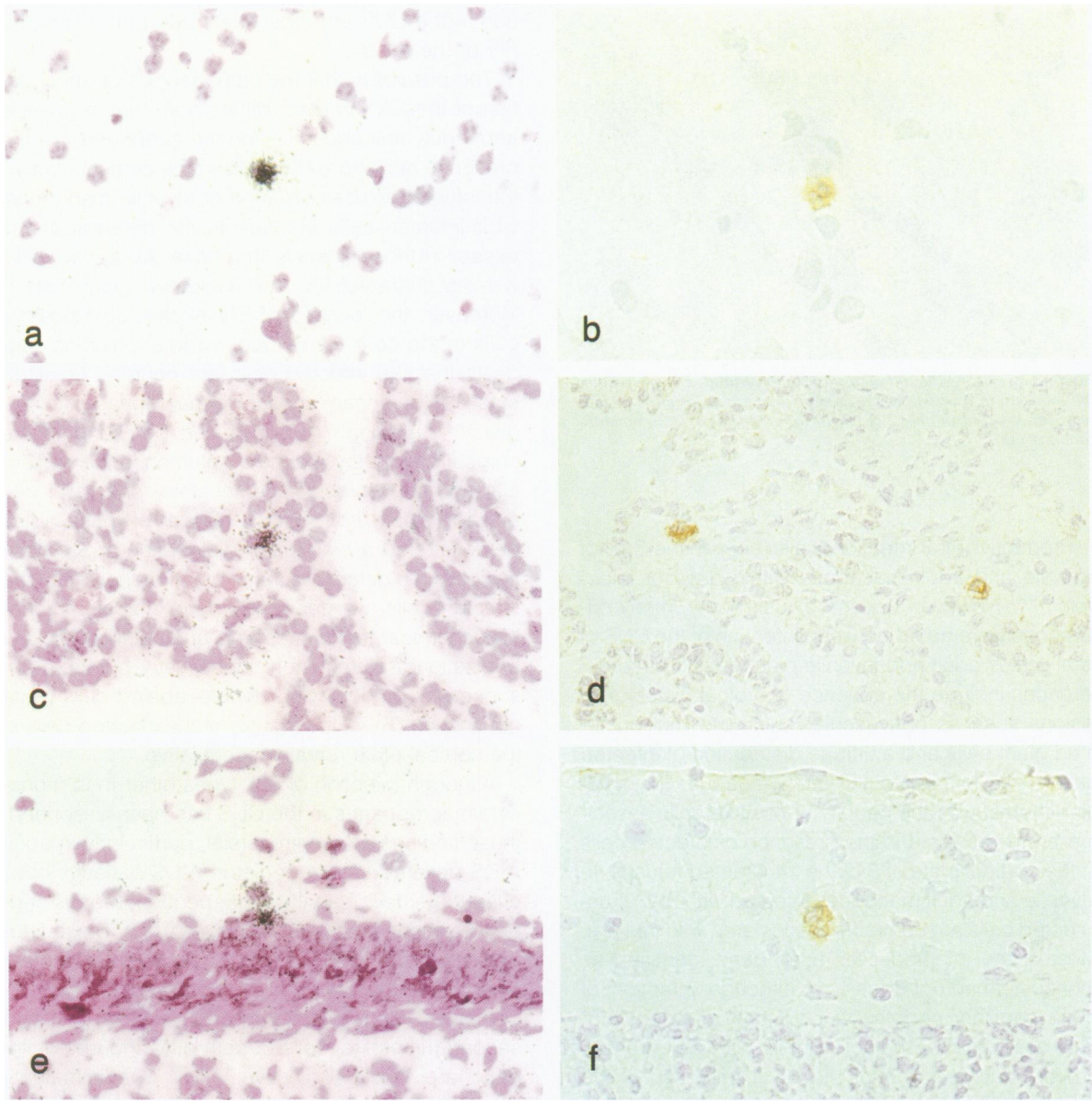

Figure 2. *Localization of SIV in fetal brain by* in situ *hybridization* (a, c, *and* e) *and immunohistochemistry* (b, d, *and* f). *Virus-positive cells were present in the cortical plate* (a *and* b), *stroma of the choroid plexus* (c *and* d), *and the meninges and external granular layer of the cerebellum* (e *and* f). *Note that virus-positive cells in the cortical plate appear to be within columns of cells* (a *and* b). *Hematoxylin counterstain* (b, d, *and* f); *H&E counterstain* (a, c, *and* e); *magnification,* ×*390.*

The cells lacked distinct morphological characteristics making identification difficult. However, 8 of the 14 animals did have *in situ* hybridization-positive cells arranged in radial columns within the cortical plate (Figure 2, a and b, and Table 2). Based on location, these cells were likely astrocytes or neurons. Virus-positive cells were also found in the external granular layer of the cerebellum, which is its primary germinative cell zone. However, positive cells were absent in the ventricular and subventricular germinative zones that give rise to the cerebral cortex. Infected cells were also surprisingly rare around vessels (found in only two animals; Table 2 and Figure 3).

Discussion

This study clearly demonstrates that *in utero* infection of fetal macaques results in persistent infection of the fetal CNS. Infected cells could be detected as early as 15 days after infection and were found in all animals regardless of GD of inoculation. The presence of detectable viral DNA, RNA, and protein indicates that the infection is productive.

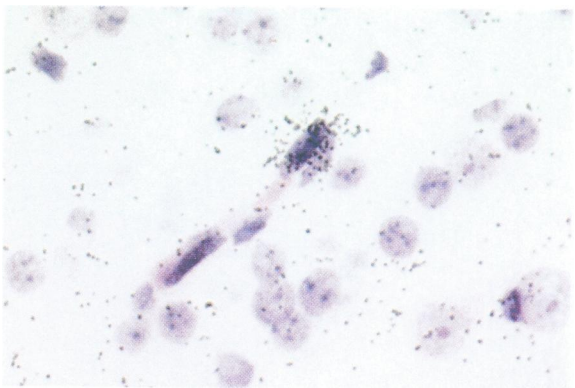

Figure 3. In situ *hybridization for SIV demonstrating a single positive cell adjacent to a vessel. A perivascular orientation for virus-positive cells was observed in only 2 of the 14 infected fetuses. H&E; magnification, ×600.*

Productive viral infection is also seen in the CNS of juvenile and adult macaques within days of infection.[22–24] However, beyond this similarity, many differences were noted between infection of the CNS of fetal *versus* adult and juvenile macaques. These differences include the absence of typical histological lesions such as perivascular cuffs and multinucleated giant cells and a unique distribution of infected cells[21,24,25] in the infected fetal macaque. Of particular interest was the paucity of positive cells associated with vessels and the presence of infected cells in the cortical plate of 8 of the 14 infected fetuses. In juvenile and adult macaques infected with SIV, virus-infected cells are commonly associated with vessels. Most of the infected cells have been identified as monocyte/macrophages,[21,22] although infection of brain capillary endothelial cells may also occur.[26] This association of infected cells with cerebral microvasculature is thought to be a reflection of the primary route of neuroinvasion by both SIV and HIV, namely, transendothelial migration of infected monocytes into the brain.[26–29] The paucity of SIV-infected cells associated with vessels in the brain of fetal macaques suggests that the pathogenesis is different during development. It is unlikely that these differences are due to different routes of infection (intravenous in juveniles and adult macaques *versus* intraperitoneal for fetal macaques) because similar doses of SIVmac have been shown to result in AIDS in juvenile and adult macaques inoculated by either the intravenous or mucosal routes.[15] Furthermore, no significant differences in the distribution of SIV-infected cells in the brain of juvenile or adult macaques inoculated by either of these routes or by direct intracerebral inoculation have been noted.[21–23,30] Thus, the differences in viral distribution in the CNS are most likely due to the immaturity of the fetus.

The possibility that the pathogenesis of SIV infection of the CNS *in utero* differs from that in juvenile and adult macaques is further supported by the presence of SIV-positive cells in the cortical plate of the infected fetuses. In adult or juvenile macaques, virus-infected cells are rare in the cerebral cortex except in those animals that have SIV encephalitis with its characteristic multinucleated giant cells.[21] Moreover, the location of SIV nucleic-acid-positive cells in the cortex arranged in radial columns suggests that the infected cells are neurons or astrocytes rather than monocyte/macrophages/microglia.[31,32] Although microglia can be found in the cortical plate, they are more common in the ventricular and subventricular germinative zones and meninges[33] (data not shown). Neither of these areas demonstrated a bias in the distribution of SIV-positive cells. To determine the immunophenotype of the infected cells, we performed double-label immunohistochemical techniques and combined *in situ* hybridization and immunohistochemistry but were unsuccessful in identifying double-labeled cells (data not shown). Thus, the identity of the infected cells in the cortical plate remains speculative.

Although infection of cell types other than monocyte/macrophages in the CNS has been described, these findings are controversial, particularly in adult AIDS patients.[34,35] In pediatric AIDS patients, however, there have been two independent reports suggesting that astrocytes are infected.[10,36] These findings in children who may have been infected *in utero* need to be considered in the context of CNS development. During gestation, particularly the first and second trimesters, cells of both glial and neural lineage are mitotically active.[31,32,37] The immaturity and active replication of these cells increase both the pool size of nucleotides that are needed for viral DNA synthesis and the level of cellular factors that are important for transcription of proviral DNA.[38] Thus, these cells may be uniquely susceptible to viral infection by SIV or HIV. This is in contrast to cells of the adult nervous system, which are mitotically inactive. These features may partially explain the apparent differences in pathogenesis of HIV-associated neurological disease between pediatric and adult AIDS patients, as well as the bimodal clinical neurological course in HIV-infected infants. Based on the rapid disease course observed in macaque fetuses infected with SIV *in utero*,[14] it seems likely that infants with rapidly progressive neurological disease were infected prenatally. The majority of pediatric AIDS patients with a slower disease course

were likely infected intrapartum or postpartum when the nervous and immune systems are more fully developed.

In summary, we have shown that direct inoculation of rhesus fetuses with SIV results in infection of the fetal CNS and that the viral distribution is unique compared with infection of older animals. This suggests that the pathogenesis of SIV- and HIV-induced neurological disease in young patients differs from that observed in juveniles and adults. Furthermore, the timing of infection of the immature host may dramatically affect the subsequent disease course. The ability to infect the fetus directly at specific gestational periods provides a powerful tool to examine these important issues.

Acknowledgments

We thank Alison Hampson for photographic assistance and Drs. Vito Sasseville and Richard Sidman for helpful comments.

References

1. Belman AL, Diamond G, Dickson D, Horoupian D, Llena J, Lantos G, Rubenstein A: Pediatric acquired immunodeficiency syndrome: neurologic syndromes. Am J Dis Child 1988, 142:29–35
2. Sharer LR, Dowling PC, Michaels J, Cook SD, Menonna J, Blumberg BM, Epstein LG: Spinal cord disease in children with HIV-1 infection: a combined biological and neuropathological study. Neuropathol Appl Neurobiol 1990, 16:317–331
3. Sharer LR, Epstein LG, Cho E-S, Joshi VV, Meyenhofer MF, Rankin LF, Petito CK: Pathologic features of AIDS encephalopathy in children: evidence for LAV/HTLV-III infection of the brain. Hum Pathol 1986, 17:271–284
4. Vazeux R, Lacroix-Ciaudo C, Blanche S, Cumont M-C, Henin D, Gray F, Boccon-Gibod L, Tardieu M: Low levels of human immunodeficiency virus replication in the brain tissue of children with severe acquired immunodeficiency syndrome encephalopathy. Am J Pathol 1992, 140:137–144
5. Belman AL: HIV-1-associated CNS disease in infants and children. Res Publ Assoc Res Nerv Ment Dis 1994, 72:289–310
6. Lyman WD, Kress Y, Kure K, Rashbaum WK, Rubinstein A, Soeiro R: Detection of HIV in fetal central nervous system tissue. AIDS 1990, 4:917–920
7. Wiley CA, Belman AL, Dickson DW, Rubenstein A, Nelson JA: Human immunodeficiency virus within the brains of children with AIDS. Clin Neuropathol 1990, 9:1–6
8. Shaw GM, Harper ME, Hahn BH, Epstein LG, Gajdusek DC, Price RW, Navia BA, Petito CK, O'Hara CJ, Cho E-S, Oleske JM, Wong-Staal F, Gallo RC: HTLV-III infection in brains of children and adults with AIDS encephalopathy. Science 1985, 227:177–182
9. Tornatore C, Chandra R, Berger JR, Major EO: HIV-1 infection of subcortical astrocytes in the pediatric central nervous system. Neurology 1994, 44:481–487
10. Saito Y, Sharer LR, Epstein LG, Michaels J, Mintz M, Louder M, Golding K, Cvetkovich TA, Blumberg BM: Overexpression of *nef* as a marker for restricted HIV-1 infection of astrocytes in postmortem pediatric central nervous tissues. Neurology 1994, 44:474–481
11. Desrosiers RC: The simian immunodeficiency viruses. Annu Rev Immunol 1990, 8:557–578
12. Gardner MB, Luciw PA: Simian immunodeficiency viruses and their relationship to the human immunodeficiency viruses. AIDS 1988, 2:S3–S10
13. Tarantal AF, Marthas ML, Gargosky SE, Otysula M, McChesney MB, Miller CJ, Hendrickx AG: Effects of viral virulence on intrauterine growth in SIV-infected fetal rhesus macaques (*Macaca mulatta*). J Acquired Immune Defic Syndr Hum Retrovirol 1995, 10:129–138
14. Tarantal AF, Marthas ML, McChesney BB, Miller CJ, Lerche NW, Pedersen NC, Hendrickx AG: Effects of SIV infection on fetal development and pregnancy outcome in the rhesus macaque (*Macaca mulatta*). Pediatr AIDS HIV Infect 1993, 4:373–380
15. Miller CJ, Marthas M, Torten J, Alexander NJ, Moore JP, Doncel GF, Hendrickx AG: Intravaginal inoculation of rhesus macaques with cell-free simian immunodeficiency virus results in persistent or transient viremia. J Virol 1994, 68:6391–6400
16. Miller CJ, Alexander NJ, Sutjipto S, Lackner AA, Hendrickx AG, Gettie A, Lowenstine LJ, Jennings M, Marx PA: Genital mucosal transmission of simian immunodeficiency virus: animal model for heterosexual transmission of human immunodeficiency virus. J Virol 1989, 63:4277–4284
17. Kestler H, Kodama T, Ringler D, Marthas M, Pedersen N, Lackner A, Regier D, Sehgal P, Daniel M, King N, Desrosiers R: Induction of AIDS in rhesus monkeys by molecularly cloned simian immunodeficiency virus. Science 1990, 248:1109–1112
18. Gibbs JS, Lackner AA, Lang SM, Simon MA, Sehgal PK, Daniel MD, Desrosiers RC: Progression to AIDS in the absence of a gene for *vpr* or *vpx*. J Virol 1995, 69:2378–2383
19. Ilyinskii PO, Daniel MD, Simon MA, Lackner AA, Desrosiers RC: The role of upstream U3 sequences in the pathogenesis of SIV-induced AIDS in rhesus monkeys. J Virol 1994, 68:5933–5944
20. Fox CH, Cottler-Fox M: *In situ* hybridization for detection of HIV RNA. Current Protocols in Immunology. Edited by JE Coligan, AM Kruisbeek, DH Margulies, EM Shevach, W Strober. New York, John Wiley and Sons, 1994, pp 12.8.1–12.8.21
21. Lackner AA, Smith MO, Munn RJ, Martfeld DJ, Gardner MB, Marx PA, Dandekar S: Localization of simian im-

munodeficiency virus in the central nervous system of rhesus monkeys. Am J Pathol 1991, 139:609–621
22. Chakrabarti L, Hurtrel M, Maire M, Vazeux R, Dormont D, Montagnier L, Hurtrel B: Early viral replication in the brain of SIV-infected rhesus monkeys. Am J Pathol 1991, 139:1273–1280
23. Lackner AA, Vogel P, Ramos RA, Kluge JD, Marthas M: Early events in tissues during infection with pathogenic (SIVmac239) and nonpathogenic (SIVmac1A11) molecular clones of simian immunodeficiency virus. Am J Pathol 1994, 145:428–439
24. Smith MO, Heyes MP, Lackner AA: Early intrathecal events in rhesus macaques (*Macaca mulatta*) infected with pathogenic or nonpathogenic molecular clones of simian immunodeficiency virus. Lab Invest 1995, 72: 547–558
25. Lackner AA: Pathology of simian immunodeficiency virus induced disease. Current Topics in Microbiology and Immunology: Simian Immunodeficiency Virus. Edited by RC Desrosiers, N Letvin. Berlin, Springer Verlag, 1994, pp 35–64
26. Mankowski JL, Spelman JP, Ressetar HG, Strandberg JD, Laterra J, Carter DL, Clements JE, Zink MC: Neurovirulent simian immunodeficiency virus replicates productively in endothelial cells of the central nervous system *in vivo* and *in vitro*. J Virol 1994, 68:8202–8208
27. Nottet HSLM, Persidsky Y, Sasseville VG, Nakuna AN, Bock P, Zhai Q, Sharer LR, McComb RD, Swindells S, Soderland C, Gendelman HE: Mechanisms for the transendothelial migration of HIV-1-infected monocytes into brain. J Immunol 1996, 156:1284–1295
28. Sasseville VG, Lane JH, Walsh D, Ringler DJ, Lackner AA: VCAM-1 expression and leukocyte trafficking to the CNS occur early in infection with pathogenic isolates of SIV. J Med Primatol 1995, 24:123–131
29. Sasseville VG, Newman WA, Lackner AA, Smith MO, Lausen NC, Beall D, Ringler DJ: Elevated vascular cell adhesion molecule-1 in AIDS encephalitis induced by simian immunodeficiency virus. Am J Pathol 1992, 141: 1021–1030
30. Boche D, Gray F, Chakrabarti L, Hurtrel M, Montagnier L, Hurtrel B: Low susceptibility of resident microglia to simian immunodeficiency virus replication during the early stages of infection. Neuropathol Appl Neurobiol 1995, 21:535–539
31. Rakic P: Mode of cell migration to the superficial layers of the monkey neocortex. J Comp Neurol 1972, 145: 61–84
32. Cameron RS, Rakic P: Glial cell lineage in the cerebral cortex: a review and synthesis. Glia 1991, 4:124–137
33. Hutchins KD, Dickson DW, Rashbaum WK, Lyman WD: Localization of morphologically distinct microglial populations in the developing human fetal brain: implications for ontogeny. Dev Brain Res 1990, 55: 95–102
34. Nuovo GJ, Gallery F, MacConnell P, Braun A: *In situ* detection of polymerase chain reaction amplified HIV-1 nucleic acids, and tumor necrosis factor-α RNA in the central nervous system. Am J Pathol 1994, 144:659–666
35. Wiley CA, Achim CL: Human immunodeficiency virus encephalitis and dementia. Ann Neurol 1995, 38:559–560
36. Tornatore C, Meyers K, Atwood W, Conant K, Major E: Temporal patterns of human immunodeficiency virus type 1 transcripts in human fetal astrocytes. J Virol 1994, 68:93–102
37. Herschkowitz N: Brain development in the fetus, neonate, and infant. Biol Neonate 1988, 54:1–19
38. Zack JA, Arrigo SJ, Weitsman SR, Go AS, Haislip A, Chen ISY: HIV-1 entry into quiescent primary lymphocytes: molecular analysis reveals a labile, latent viral structure. Cell 1990, 61:213–222