Decreased Expression of MAP-2 and GAD in the Brain of Cats Infected with Feline Immunodeficiency Virus

TIRTHA RAJ KOIRALA,¹ KEIKO NAKAGAKI,¹ TAKUO ISHIDA,² SHUKO NONAKA,²
SATOKO MORIKAWA² and TAKESHI TABIRA^{1,3}

¹*Division of Demyelinating Disease and Aging, National Institute of Neuroscience, Tokyo 187-8502,*

²*Nippon Veterinary and Animal Science University, Tokyo 180-8602, and*

³*National Institute for Longevity Sciences, Aichi 474-8522*

KOIRALA, T.R., NAKAGAKI, K., ISHIDA, T., NONAKA, S., MORIKAWA, S. and TABIRA, T. *Decreased Expression of MAP-2 and GAD in the Brain of Cats Infected with Feline Immunodeficiency Virus.* Tohoku J. Exp. Med., 2001, **195**(3), 141–151 — HIV-1 infection is often complicated by the dysfunction of central nervous system (CNS). Degenerative neuronal changes as well as neuronal loss have been documented in individuals with acquired immunodeficiency syndrome. Feline immunodeficiency virus (FIV) causes similar CNS manifestation and FIV infected cats provide an animal model for human immunodeficiency virus infection in humans. In this study, we examined the brain of FIV-infected cats and controls with immunohistochemical techniques using antibodies to microtubule-associated protein 2 (MAP-2) and glutamic acid decarboxylase (GAD). We found a significant decrease in expression of MAP-2 and GAD in neurons of infected animals compared to controls. In contrast, the expression of neurofilaments and glial fibrillary acidic protein was rather increased. The changes observed in the brain were similar to those seen in humans undergoing the normal aging process as well as those suffering from neurological diseases like Alzheimer's disease and other dementing disorders. These changes in the feline brain give insight into the deleterious effects of FIV on the CNS. ——— AIDS; FIV; glutamic acid decarboxylase; HIV-1; microtubule-associated protein

© 2001 Tohoku University Medical Press

Received January 29, 2001 ; revision accepted for publication November 2, 2001.

Address for reprints: Takeshi Tabira, National Institute for Longevity Sciences, 36-3 Gengo, Morioka, Obu, Aichi 474-8522, Japan.

e-mail: tabira@nils.go.jp

Feline immunodeficiency virus (FIV) was first discovered in 1986 as a cause of immune deficiency and neurological diseases in domestic cats (Pedersen et al. 1987). FIV and human immunodeficiency virus-1 (HIV-1) share many structural and biochemical properties, and elicit a broad spectrum of similar neurological disease/deficit (Elder and Phillips 1993; Podell et al. 1993; Phillips et al. 1994; Henriksen et al. 1995). HIV-1- and FIV-infected brains also share many similar neuropathological changes, including neuronal loss and damage, which are common sequelae to infection (Gray et al. 1991; Gendelman and Tardieu 1994; Dewhurst et al. 1996; Meeker 1997). Yet, there is a lack of compelling evidence how the virus damages neurons. There is some evidence that HIV mediates its effect on neurons through an indirect mechanism rather than a direct one (Gendelman and Tardieu 1994; Lipton 1994a; Dewhurst et al. 1996). Numerous studies have shown that microtubule associated protein 2 (MAP-2) and neurofilament protein are sensitive markers for neurodegenerative diseases in humans as well as in animals (Schlaepfer 1987; Hof et al. 1992; Masliah et al. 1993; Price 1994; Jacobson et al. 1997; Poli et al. 1997; Montgomery et al. 1999).

The purpose of this study is to determine whether or not dendritic abnormalities similar to those reported in cases of HIV encephalitis (Masliah et al. 1992b) are present in cats infected with FIV. Studies of the glutamic acid decarboxylase (GAD) profile in neurons have not yet been reported for FIV-infected brains and this study also aimed to determine this profile. Using immunohistochemical methods with these antibodies we observed abnormal neuronal profiles in the FIV-infected brains compared to the controls. The feline system can thus be proposed as a useful model for testing therapeutic strategies to combat the effects of HIV-1 in the central nervous system (CNS).

MATERIALS AND METHODS

FIV infection

The FIV used is the Petalma strain which was adapted to Crandle feline kidney cells. The experimental protocol was approved by the Ethics Review Committee for Animal Experimentation of the Nippon Veterinary Medical School. The virus was inoculated intracisterally into 3 mongrel adult cats (4–6 years old) at a dose of 12 000 reverse transcriptase units per animal. For anesthesia, isoflurane was used. Each animal was kept in a cage sized $38 \times 47 \times 50$ cm, bed material was daily changed, and animals were fed with fresh food and water every day. FIV-infected cats showed lymphadenopathy accompanied by episodes of neutropenia, lymphopenia, pyrexia, anorexia, rhinitis, dullness, cachexia and behavioral abnormalities.

Brain sections

Animals were deeply anesthetized and 2 were killed seven months after and one was one year after FIV inoculation. Three FIV-positive and three age-matched control brains were fixed in 4% formalin and tissues from the cortex of the frontal, parietal, temporal and occipital lobes and the hippocampus were embedded in paraffin. Four micrometer thick sections were cut and used for hematoxylin and eosin (H & E) and immunohistochemical staining.

Immunohistochemistry

Immunohistochemistry was performed using the avidin-biotin-peroxidase complex (ABC) method with a Vectastain ABC kit (Vector, Burlingame, CA, USA), according to the manufacturer's instructions. Briefly, the sections were deparaffinized in xylene and rehydrated through graded alcohol concentrations (100, 95, 80 and 70%). After quenching the endogenous peroxidase with 0.3% H_2O_2 the slides were incubated with diluted normal

serum for 20 minutes and then at 4°C overnight with the primary antibodies at different dilutions as described below. The next day, the sections were incubated first with biotinylated secondary antibody and then with Vectastain ABC reagent at room temperature for 30 minutes each. Color development using diaminobenzidine was performed until the desired degree of staining was achieved. All tissue sections were then counterstained with Mayer's hematoxylin and mounted with Gel/Mount (Biomed, Foster City, CA, USA). For microtubule association protein 2 (MAP-2) immunohistochemistry, the sections were subjected to 10 minutes of microwave pretreatment in citrate buffer (pH 6) before they were placed in diluted normal serum for 20 minutes in the above procedure. Antibodies used were the mouse monoclonal antibodies to MAP-2 ($>5 \mu\text{g/ml}$; Boehringer Mannheim, PA, USA), which identifies neuronal cell bodies and dendrites, GAD⁶⁵ ($1 \mu\text{g/ml}$ or 1:100; Chemicon International Inc., CA, USA), which identifies the glutamic acid decarboxylase present in the neurons, and SMI 32 (1:1000; Sternberger Monoclonals, MD, USA), which identifies neurofilament, and a rabbit polyclonal antibody to glial fibrillary acidic protein (GFAP) (1:5; Dako, Carpinteria, CA, USA).

Control experiments were carried out by replacing the primary antibody with the same concentration of irrelevant immunoglobulins of the same class IgG isotype or 0.5% bovine serum albumin/Tris buffered saline.

Quantitation

Cells stained with MAP-2 and GAD were counted using a Compound Olympus microscope at a magnification of $\times 40$. A total of 15 slides from uninfected and 15 slides from infected cats (five slides from each cat) were selected for counting. Stained cells were counted in five adjacent fields in each slide in blind.

RESULTS

Initial H & E staining showed pathological changes including gliosis, white matter pallor, meningitis and perivascular cuffing. Some glial nodules were also observed (data not shown). Immunohistochemical analysis showed that MAP-2-positive cells were observed in the neurons and their dendrites of both the FIV-infected and control sections. The staining was localized in the perikarya, usually with perinuclear localization extending up to some proximal regions of the neuronal cell processes. Expression levels of MAP-2 were much stronger in the neurons of the uninfected brain (Fig. 1A) and weaker in the infected brain (Fig. 1B). At the same time, in the FIV-infected brains, the number of stained neurons and their processes was markedly reduced. Some of the neurons were weakly stained, with no or very little staining of dendrites.

Immunostaining of GAD was observed in most of the cortical neurons of the uninfected brain (Fig. 2A), but only half or less than half of the neurons were stained with anti-GAD antibody in the FIV-infected brain (Fig. 2B). This difference in staining pattern was more marked in the cat that survived for one year. In the other two infected cats, the staining pattern of GAD immunoreactivity was identical to that in uninfected control cats, but the level of expression decreased compared to that of controls.

Contrary, the expression of neurofilament stained with SMI 32 was slightly higher in FIV-infected brains than in the uninfected brains (Figs. 3A and 3B). Similarly, GFAP expression was higher in the FIV-infected brains (Figs. 4A and 4B), although the number of astrocytes was not increased. Control staining of the same cat brain sections with identical dilutions of isotype-matched CNS-irrelevant immunoglobulins or without primary antibodies were negative (data not shown).

A quantitative analysis showed that MAP-

2-positive neurons in the infected brain were 45–55% less than those in the uninfected brain and GAD-positive neurons were 40–50% in a one-year-post-infection cat compared to the stained neurons in the control brain.

DISCUSSION

Neuronal pathology in AIDS is of interest in relation to cognitive impairment in AIDS patients and from the broader perspective of the pathogenesis of neurodegeneration. Neuronal loss and GAD activity have already been studied in humans and rhesus monkeys with AIDS, respectively, (Masliah et al. 1992b; Koutsilieri et al. 1999) but have not yet been studied in FIV-infected animals.

Our results parallel those found in humans and macaques infected with HIV-1 and simian immunodeficiency virus (SIV), respectively (Masliah et al. 1992b; Montgomery et al. 1999). Strong expression of feline MAP-2 showed a somatodendritic pattern in controls, but there were distinct differences noticeable in infected brains. The perikaryal staining was less clear in the two cats infected for a relatively shorter time, and this was associated with slight dendritic irregularity. A marked decrease in the intensity of MAP-2 immunoreactivity of neurons and their dendrites was seen in the cat that survived longer. This result closely resembles those described in AIDS patients and SIV-infected macaques (Masliah et al. 1992b; Montgomery et al. 1999). Loss of MAP-2 immunoreactivity is also seen in other pathological conditions like cerebral ischaemia (Matesic and Lin 1994), Retts syndrome (Kaufmann et al. 1995) and administration of serotonin-antagonists (Whitaker-Azmitia et al. 1995) or neurotoxins (Koczyk 1994).

The pathogenesis of the dendritic loss is unclear, but such loss is a feature of many other neurological disorders (Jagadha and Becker 1989) including chronic murine retroviral encephalitis (Nagra et al. 1994), scrapie in

hamsters (Hogan et al. 1987), mental retardation (Ferrer and Gullotta 1990; Huttenlocher 1991), epilepsy (Multani et al. 1994), Alzheimer's disease (Ferrer and Gullotta 1990) and chronic experimental alcohol consumption (Lescaudron et al. 1989). The changes appeared to be present over the entire dendritic extent of all spine-bearing neurons present in the cortical blocks examined, suggesting a diffuse process. The insult leading to dendritic loss is likely to be acting locally, but the possibility that it arises secondary to axonal pathology as reported in pre-AIDS (Park et al. 1996) can not be excluded.

In some circumstances the loss appears to be mediated by an excitotoxic mechanism, possibly via activation of calpain I caused by increased levels of free intracellular calcium (Felipo et al. 1993). That a similar process involving excitotoxicity and loss of calcium homeostasis is occurring in the neuronal pathogenesis of HIV and SIV infection is supported by a wealth of *in vitro* studies of the effects of viral proteins and inflammatory mediators upon neurons (Lipton 1994b).

Numerous studies have demonstrated that HIV envelope protein gp 120 may be neurotoxic or that higher levels of it, or other markers of virus load, are associated with more severe CNS pathology (Brenneman et al. 1988; Lipton et al. 1991; Masliah et al. 1992a, 1995; Hill et al. 1993; Royal et al. 1994; Singer et al. 1994; Bagetta et al. 1995). Recently, low-density lipoprotein receptor related protein-mediated uptake of Tat protein was also shown neurotoxic (Liu et al. 2000).

A variety of inflammatory mediators have been proposed as possible neurotoxic intermediators (Heyes et al. 1992; Giuliani et al. 1993, 1996; Gelbard et al. 1994; Bukrinsky et al. 1995). These mediators have most often been considered in the context of HIV infection of macrophages but CNS immune activation from whatever cause might be expected to give rise to or exacerbate neurotoxicity. For

instance, there is growing evidence supporting a protective role for anti-inflammatory agents in Alzheimer's disease (McGeer et al. 1996) and aspirin can protect from excitotoxic damage (Grilli et al. 1996). In view of uncertain role of gp 120 in the neuropathogenesis of AIDS, the existence of neurotoxic inflammatory processes independent of CNS production of gp 120 or other viral protein(s) can not be excluded. Similar mechanisms could operate in the feline brain after FIV infection.

To our knowledge, there have been no previous reports on the expression of GAD in feline brain cells. Our results with the antibody to GAD⁶⁵ were new findings about FIV-infected brains. GAD is an enzyme that catalyzes the production of gamma aminobutyric acid (GABA), an inhibitory neurotransmitter in the brain, from glutamate. In the infected brain immunoreactivity with this antibody was markedly reduced compared to that in the uninfected brain, and the reduction was progressively more marked with increasing duration of infection. This indicates the loss of GAD, and implies a parallel decrease in the GABA from the neurons and nerve terminals (synapses). There is substantial evidence from experimental and clinical studies that the GABA concentration in the CSF may reflect central GABA-ergic activity (Enna et al. 1977a, b; Koutsilieri et al. 1999), that in turn reflects GAD activity. A reduction in the GABA-ergic function has been postulated to underlie seizure pathogenesis (Loscher and Schwark 1985). Seizures have been reported in the HIV and SIV models (Rausch et al. 1994) but there are no available reports yet regarding GABA/GAD concentrations in HIV or SIV. The reduction in GAD in our study was 40–50% at one year postinfection compared to the normal level but we did not study GABA levels. We also did not study whether the animals in this study suffered from seizures or not. However, as little as 30% decreases of GABA in brain nerve terminals have been demonstrated to impair the function-

ing of this inhibitory transmitter, leading to seizures (Wood et al. 1979). This is in line with the decrease of GAD activity in the SIV-infected brain, and similar effects can be postulated in the FIV-infected brain as well.

The results obtained with antibodies to GFAP and SMI 32 in our study were similar to those obtained in previous studies with these antibodies in FIV-infected feline brain tissue (Jacobson et al. 1997; Poli et al. 1997). In brief, GFAP immunoreactivity was markedly higher in infected than in uninfected animals, suggesting astrocytes are activated. Similarly, a significant increase in the SMI 32 immunoreactivity of the neurons was observed in FIV-infected brains compared to controls.

In conclusion, dendritic abnormality, downregulation of MAP-2 and GAD, accumulation of neurofilament, and activation of astrocytes characterize the pathological features of FIV-infected brain.

Acknowledgments

This work was supported by a grant (AIDS) from the Ministry of Health, Welfare and Labor, Japan.

References

- Bagetta, G., Corasaniti, M.T., Berliocchi, L., Navarra, M., Finazzi-Agro, A. & Nistico, G. (1995) HIV-1 gp120 produces DNA fragmentation in the cerebral cortex of rat. *Biochem. Biophys. Res. Commun.*, **211**, 130–136.
- Brenneman, D.E., Westbrook, G.L., Fitzgerald, S.P., Ennist, D.L., Elkins, K.L., Ruff, M.R. & Pert, C.B. (1988) Neuronal cell killing by the envelope protein of HIV and its prevention by vasoactive intestinal peptide. *Nature*, **335**, 639–642.
- Bukrinsky, M.I., Nottet, H.S., Schmidtayerova, H., Dubrovsky, L., Flanagan, C.R., Mullins, M.E., Lipton, S.A. & Gendelman, H.E. (1995) Regulation of nitric oxide synthase activity in human immunodeficiency virus type 1 (HIV-1)-infected monocytes: implications for HIV-associated neurological disease. *J. Exp. Med.*, **181**, 735–745.

- Dewhurst, S., Gelbard, H.A. & Fine, S.M. (1996) Neuropathogenesis of AIDS. *Mol. Med. Today*, **2**, 16-23.
- Elder, J.H. & Phillips, T.R. (1993) Molecular properties of feline immunodeficiency virus (FIV). *Infect. Agents Dis.*, **2**, 361-374.
- Enna, S.J., Stern, L.Z., Wastek, G.J. & Yamamura, H.I. (1977a) Cerebrospinal fluid gamma-aminobutyric acid variations in neurological disorders. *Arch. Neurol.*, **34**, 683-685.
- Enna, S.J., Wood, J.H. & Snyder, S.H. (1977b) Gamma-Aminobutyric acid (GABA) in human cerebrospinal fluid: radioreceptor assay. *J. Neurochem.*, **28**, 1121-1124.
- Felipo, V., Grau, E., Minana, M.D. & Grisolia, S. (1993) Activation of NMDA receptor mediates the toxicity of ammonia and the effects of ammonia on the microtubule-associated protein MAP-2. *Adv. Exp. Med. Biol.*, **341**, 83-93.
- Ferrer, I. & Gullotta, F. (1990) Down's syndrome and Alzheimer's disease: dendritic spine counts in the hippocampus. *Acta Neuropathol. (Berl.)*, **79**, 680-685.
- Gelbard, H.A., Nottet, H.S., Swindells, S., Jett, M., Dzenko, K.A., Genis, P., White R., Wang L., Choi Y.B. & Zhang D. (1994) Platelet-activating factor: a candidate human immunodeficiency virus type 1-induced neurotoxin. *J. Virol.*, **68**, 4628-4635.
- Gendelman, H.E. & Tardieu, M. (1994) Macrophages/microglia and the pathophysiology of CNS injuries in AIDS. *J. Leukoc. Biol.*, **56**, 387-388.
- Giulian, D., Wendt, E., Vaca, K. & Noonan, C.A. (1993) The envelope glycoprotein of human immunodeficiency virus type 1 stimulates release of neurotoxins from monocytes. *Proc. Natl. Acad. Sci. USA*, **90**, 2769-2773.
- Giulian, D., Yu, J., Li, X., Tom, D., Li, J., Wendt, E., Lin, S.N., Schwarcz, R. & Noonan, C. (1996) Study of receptor-mediated neurotoxins released by HIV-1-infected mononuclear phagocytes found in human brain. *J. Neurosci.*, **16**, 3139-3153.
- Gray, F., Haug, H., Chimelli, L., Geny, C., Gaston, A., Scaravilli, F. & Budka, H. (1991) Prominent cortical atrophy with neuronal loss as correlate of human immunodeficiency virus encephalopathy. *Acta Neuropathol. (Berl.)*, **82**, 229-233.
- Grilli, M., Pizzi, M., Memo, M. & Spano, P. (1996) Neuroprotection by aspirin and sodium salicylate through blockade of NF-kappa B activation. *Science*, **274**, 1383-1385.
- Henriksen, S.J., Prospero-Garcia, O., Phillips, T.R., Fox, H.S., Bloom, F.E. & Elder, J.H. (1995) Feline immunodeficiency virus as a model for study of lentivirus infection of the central nervous system. *Curr. Top. Microbiol. Immunol.*, **202**, 167-186.
- Heyes, M.P., Saito, K., Crowley, J.S., Davis, L.E., Demitrack, M.A., Der, M., Dilling, L.A., Elia, J., Kruesi, M.J. & Lackner, A. (1992) Quinolinic acid and kynurenine pathway metabolism in inflammatory and non-inflammatory neurological disease. *Brain*, **115**, 1249-1273.
- Hill, J.M., Mervis, R.F., Avidor, R., Moody, T.W. & Brenneman, D.E. (1993) HIV envelope protein-induced neuronal damage and retardation of behavioral development in rat neonates. *Brain Res.*, **603**, 222-233.
- Hof, P.R., Bouras, C., Buee, L., Delacourte, A., Perl, D.P. & Morrison, J.H. (1992) Differential distribution of neurofibrillary tangles in the cerebral cortex of dementia pugilistica and Alzheimer's disease cases. *Acta Neuropathol.*, **85**, 23-30.
- Hogan, R.N., Baringer, J.R. & Prusiner, S.B. (1987) Scrapie infection diminishes spines and increases varicosities of dendrites in hamsters: a quantitative Golgi analysis. *J. Neuropathol. Exp. Neurol.*, **46**, 461-473.
- Huttenlocher, P.R. (1991) Dendritic and synaptic pathology in mental retardation. *Pediatr. Neurol.*, **7**, 79-85.
- Jacobson, S., Henriksen, S.J., Prospero-Garcia, O., Phillips, T.R., Elder, J.H., Young, W.G., Bloom, F.E. & Fox, H.S. (1997) Cortical neuronal cytoskeletal changes associated with FIV infection. *J. Neurovirol.*, **3**, 283-289.
- Jagadha, V. & Becker, L.E. (1989) Dendritic pathology: an overview of Golgi studies in man. *Can. J. Neurol. Sci.*, **16**, 41-50.
- Kaufmann, W.E., Naidu, S. & Budden, S. (1995) Abnormal expression of microtubule-associated protein 2 (MAP-2) in neocortex in Rett syndrome. *Neuropediatrics*, **26**, 109-113.
- Koczyk, D. (1994) Differential response of microtubule-associated protein 2 (MAP-2) in rat hippocampus after exposure to trimethyltin (TMT): an immunocytochemical study. *Acta Neurobiol. Exp. (Warsz.)*, **54**, 55-58.

- Koutsilieris, E., Sopper, S., Heinemann, T., Scheller, C., Lan, J., Stahl-Hennig, C., ter Meulen, V., Riederer, P. & Gerlach, M. (1999) Involvement of microglia in cerebrospinal fluid glutamate increase in SIV-infected rhesus monkeys (*Macaca mulatta*). *AIDS Res. Hum. Retroviruses*, **15**, 471-477.
- Lescaudron, L., Jaffard, R. & Verna, A. (1989) Modifications in number and morphology of dendritic spines resulting from chronic ethanol consumption and withdrawal: a Golgi study in the mouse anterior and posterior hippocampus. *Exp. Neurol.*, **106**, 156-163.
- Lipton, S.A., Sucher, N.J., Kaiser, P.K. & Dreyer, E.B. (1991) Synergistic effects of HIV coat protein and NMDA receptor-mediated neurotoxicity. *Neuron*, **7**, 111-118.
- Lipton, S.A. (1994a) HIV-related neuronal injury. Potential therapeutic intervention with calcium channel antagonists and NMDA antagonists. *Mol. Neurobiol.*, **8**, 181-196.
- Lipton, S.A. (1994b) Neuronal injury associated with HIV-1 and potential treatment with calcium-channel and NMDA antagonists. *Dev. Neurosci.*, **16**, 145-151.
- Liu, Y., Jones, M., Hingtgen, C.M., Bu, G., Laribee, N., Tanzi, R.E., Moir, R.D., Nath, A. & He, J.J. (2000) Uptake of HIV-1 Tat protein mediated by low-density lipoprotein receptor-related protein disrupts the neuronal metabolic balance of the receptor ligands. *Nature Med.*, **6**, 1380-1387.
- Loscher, W. & Schwark, W.S. (1985) Evidence for impaired GABAergic activity in the substantia nigra of amygdaloid kindled rats. *Brain Res.*, **339**, 146-1450.
- Masliah, E., Achim, C.L., Ge, N., DeTeresa, R., Terry, R.D. & Wiley, C.A. (1992a) Spectrum of human immunodeficiency virus-associated neocortical damage. *Ann. Neurol.*, **32**, 321-329.
- Masliah, E., Ge, N., Morey, M., DeTeresa, R., Terry, R.D. & Wiley, C.A. (1992b) Cortical dendritic pathology in human immunodeficiency virus encephalitis. *Lab. Invest.*, **66**, 285-291.
- Masliah, E., Mallory, M., Hansen, L., Alford, M., DeTeresa, R. & Terry, R. (1993) An antibody against phosphorylated neurofilaments identifies a subset of damaged association axons in Alzheimer's disease. *Am. J. Pathol.*, **142**, 871-882.
- Masliah, E., Ge, N., Achim, C.L. & Wiley, C.A. (1995) Differential vulnerability of calbindin-immunoreactive neurons in HIV encephalitis. *J. Neuropathol. Exp. Neurol.*, **54**, 350-357.
- Matesic, D.F. & Lin, R.C. (1994) Microtubule-associated protein 2 as an early indicator of ischemia-induced neurodegeneration in the gerbil forebrain. *J. Neurochem.*, **63**, 1012-1020.
- McGeer, P.L., Schulzer, M. & McGeer, E.G. (1996) Arthritis and anti-inflammatory agents as possible protective factors for Alzheimer's disease: a review of 17 epidemiologic studies. *Neurology*, **47**, 425-432.
- Meeker, R.B., Thiede, B.A., Hall, C., English, R. & Tompkins, M. (1997) Cortical cell loss in asymptomatic cats experimentally infected with feline immunodeficiency virus. *AIDS Res. Hum. Retroviruses*, **13**, 1131-1140.
- Montgomery, M.M., Dean, A.F., Taffs, F., Stott, E.J., Lantos, P.L. & Luthert, P.J. (1999) Progressive dendritic pathology in cynomolgus macaques infected with simian immunodeficiency virus. *Neuropathol. Appl. Neurobiol.*, **25**, 11-19.
- Multani, P., Myers, R.H., Blume, H.W., Schomer, D.L. & Sotrel, A. (1994) Neocortical dendritic pathology in human partial epilepsy: a quantitative Golgi study. *Epilepsia*, **35**, 728-736.
- Nagra, R.M., Masliah, E., Burrola, P.G. & Wiley, C.A. (1994) Neocortical pathology in chronic murine retroviral encephalitis. *J. Neuropathol. Exp. Neurol.*, **53**, 572-581.
- Park, J.S., Bateman, M.C. & Goldberg, M.P. (1996) Rapid alterations in dendrite morphology during sublethal hypoxia or glutamate receptor activation. *Neurobiol. Dis.*, **3**, 215-227.
- Pedersen, N.C., Ho, E.W., Brown, M.L. & Yamamoto, J.K. (1987) Isolation of a T-lymphotropic virus from domestic cats with an immunodeficiency-like syndrome. *Science*, **235**, 790-793.
- Phillips, T.R., Prospero-Garcia, O., Puaoli, D.L., Lerner, D.L., Fox, H.S., Olmsted, R.A., Bloom, F.E., Henriksen, S.J. & Elder, J.H. (1994) Neurological abnormalities associated with feline immunodeficiency virus infection. *J. Gen. Virol.*, **75**, 979-987.
- Podell, M., Oglesbee, M., Mathes, L., Krakowka, S., Olmstead, R. & Lafrado, L. (1993) AIDS-associated encephalopathy with experimental

- feline immunodeficiency virus infection. *J. Acquir. Immune. Defic. Syndr.*, **6**, 758-771.
- Poli, A., Abramo, F., Di Iorio, C., Cantile, C., Carli, M.A., Pollera, C., Vago, L., Tosoni, A. & Costanzi, G. (1997) Neuropathology in cats experimentally infected with feline immunodeficiency virus: a morphological, immunocytochemical and morphometric study. *J. Neurovirol.*, **3**, 361-368.
- Price, R.W. (1994) Understanding the AIDS dementia complex (ADC). The challenge of HIV and its effects on the central nervous system. *Res. Publ. Assoc. Res. Nerv. Ment. Dis.*, **72**, 1-45.
- Rausch, D.M., Heyes, M.P., Murray, E.A., Lendvay, J., Sharer, L.R., Ward, J.M., Rehm, S., Nohr, D., Weihe, E. & Eiden, L.E. (1994) Cytopathologic and neurochemical correlates of progression to motor/cognitive impairment in SIV-infected rhesus monkeys. *J. Neuropathol. Exp. Neurol.*, **53**, 165-175.
- Royal, W., III, Selnes, O.A., Concha, M., Nance-Sproson, T.E. & McArthur, J.C. (1994) Cerebrospinal fluid human immunodeficiency virus type 1 (HIV-1) p24 antigen levels in HIV-1-related dementia. *Ann. Neurol.*, **36**, 32-39.
- Schlaepfer, W.W. (1987) Neurofilaments: structure, metabolism and implications in disease. *J. Neuropathol. Exp. Neurol.*, **46**, 117-129.
- Singer, E.J., Syndulko, K., Fahy-Chandon, B.N., Shapshak, P., Resnick, L., Schmid, P., Conrad, A.J. & Tourtellotte, W.W. (1994) Cerebrospinal fluid p24 antigen levels and intrathecal immunoglobulin G synthesis are associated with cognitive disease severity in HIV-1. *AIDS*, **8**, 197-204.
- Whitaker-Azmitia, P.M., Borella, A. & Raio, N. (1995) Serotonin depletion in the adult rat causes loss of the dendritic marker MAP-2. A new animal model of schizophrenia. *Neuropsychopharmacology*, **12**, 269-272.
- Wood, J.D., Russell, M.P., Kurylo, E. & Newstead, J.D. (1979) Stability of synaptosomal GABA levels and their use in determining the in vivo effects of drugs: convulsant agents. *J. Neurochem.*, **33**, 61-68.

Illustrations follow

- Fig. 1. Immunostaining of neurons and their processes for MAP-2 in normal uninfected brain (A) and FIV-infected brain (B), $\times 200$. Stronger immunoreactivity of MAP-2 in neurons and dendrites can be seen in normal brain than in FIV-infected brain where the number of stained neurons and dendrites is reduced and the intensity is relatively weaker.
- Fig. 2. Immunostaining of the cortical neurons with anti-GAD in normal uninfected brain (A) and FIV-infected brain (B), $\times 200$. The number of neurons stained with anti-GAD is markedly reduced in the FIV-infected brain.
- Fig. 3. Photomicrographs of SMI 32-immunostained neurons in normal uninfected brain (A) showing faint staining within cell bodies and dendrites, and in FIV-infected brain (B) showing frequent and strongly stained cells.
- Fig. 4. GFAP immunoreactivity in the cortical matter of normal uninfected brain (A) and FIV-infected brain (B). The staining is much stronger in the FIV-infected brain.