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*Invited Review*

# Cell types in the central nervous system infected by murine retroviruses: implications for the mechanisms of neurodegeneration

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**Summary.** Retroviruses are an important cause of neurologic disease in humans but the pathogenic mechanisms are poorly understood. To delineate pathogenic mechanisms in any neurologic disease in humans is extremely difficult and will continue to rely on the use of animal models. This review presents several murine models to study the pathogenic mechanisms of neurodegenerative disease which manifest noninflammatory spongiform lesions in the CNS. The cell types in the CNS infected by these murine retroviruses and their role in disease induction are discussed.

**Key words:** Retroviruses, Nervous system, Neurodegeneration, Mechanisms

## Introduction

A number of murine leukemia viruses (MuLVs) have been shown to invade the nervous system and cause neurologic diseases (Table 1). Despite the variations in virus strains and mouse strains used by the various groups of researchers, most of the neurologic diseases induced by these neuropathogenic MuLVs show a predictable clinical and histopathological profile, albeit the incidence, latency, and severity of the disease may vary. The most common clinical feature is a progressive hind-limb paralysis and the most pronounced pathologic feature is spongiform neurodegeneration in the absence of inflammation. Although the viral envelope protein has been shown to play a critical role in pathogenesis of these neurologic diseases, the mechanisms underlying the neurologic dysfunction are unclear, and several hypothesis have been proposed (Wong, 1990; Jolicoeur et al., 1991, 1992; Wong and Yuen, 1992; Gonzalez-Scarano et al., 1995). Most controversial is whether neuronal cell death is the direct result of virus infection or is caused indirectly by viral infection of glial and endothelial cells. In this review we will briefly summarize the principal histopathologic features of the neurodegenerative disease, placing special emphasis on the cell types infected and their roles in the neuropathogenesis induced by these MuLVs.

A wealth of information has already accumulated on the neurodegeneration induced by MuLVs (for review see Gardner, 1985, 1991; Hoffman et al., 1988; Portis, 1990; Wong, 1990; Jolicoeur et al., 1991, 1992; Wong and Yuen, 1992; Wiley and Gardner, 1993; Gonzalez-Scarano et al., 1995). Because the murine models offer a relatively convenient and rapid system in which to study pathophysiology in the central nervous system (CNS), murine studies should hasten our understanding of the pathogenic mechanism involved in retrovirus-induced neurodegenerative diseases, which in turns should increase our understanding of human neurodegenerative diseases such as human immunodeficiency virus (HIV)-induced dementia, Alzheimer's disease, amyotrophic lateral sclerosis (ALS), Cruetzfeldt-Jacob disease (CJD), and prion-induced neurodegeneration.

## Histopathologic features

The prominent histopathologic feature shared by these MuLVs is the extensive vacuolation within the neuropil which begins as small vacuoles in the perivascular and perineuronal regions. These vacuoles increase in size and number as the disease progresses. Fig.1A and B, illustrate respectively an early and a late stage of spongiform changes within the CNS of a ts1 MoMuLV-infected FVB/N mouse. Apoptotic neurons (Fig. 1B) with cellular and nuclear condensation are also consistently present (Stoica and Wong, unpublished data).

Ultrastructural studies in several models have shown that vacuoles arise predominantly at postsynaptic

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terminals in diseased areas (Lynch et al., 1991; Swarz et al., 1981; Nagra et al., 1992; Stoica et al., 1993). An example of this vacuolar change at the postsynaptic site is shown in a ts1 MoMuLV-infected FBV/N mouse 15 days postinjection (Fig. 2). These findings suggest that postsynaptic sites (neuronal dendrites and soma) are the primary targets for the induction of degenerative changes.

The spongiform lesions induced by MuLV are remarkably similar to the lesion seen in the spongiform encephalopathy in scrapie and CJD (Gajdusek, 1992; Prusiner, 1992). ALS, and Alzheimer's disease (Smith et al., 1987). These spongiform lesions are also similar to the heritable spongiform encephalopathy associated with gray-tremor mutation gt/gt in NSF mice (Hoffman et al., 1987). Furthermore, these spongiform lesions are similar to the HIV-related vacuolar myelopathy found in some patients who have AIDS (for review, see Conzález-Scarano et al., 1995).

From the peripheral target organs, primarily the spleen and/or the thymus, the virus spreads to the CNS. In general, the time of disease onset depends on how fast the virus reaches the CNS and the rate of virus replication within the CNS. The severity of the spongiform changes also correlates with the titers of the virus in the CNS, indicating a direct effect of viral replication within the CNS on disease development (Brooks et al., 1980; Wong et al., 1985, 1991; Lynch et al., 1991).

Although the ultimate distribution of spongiform

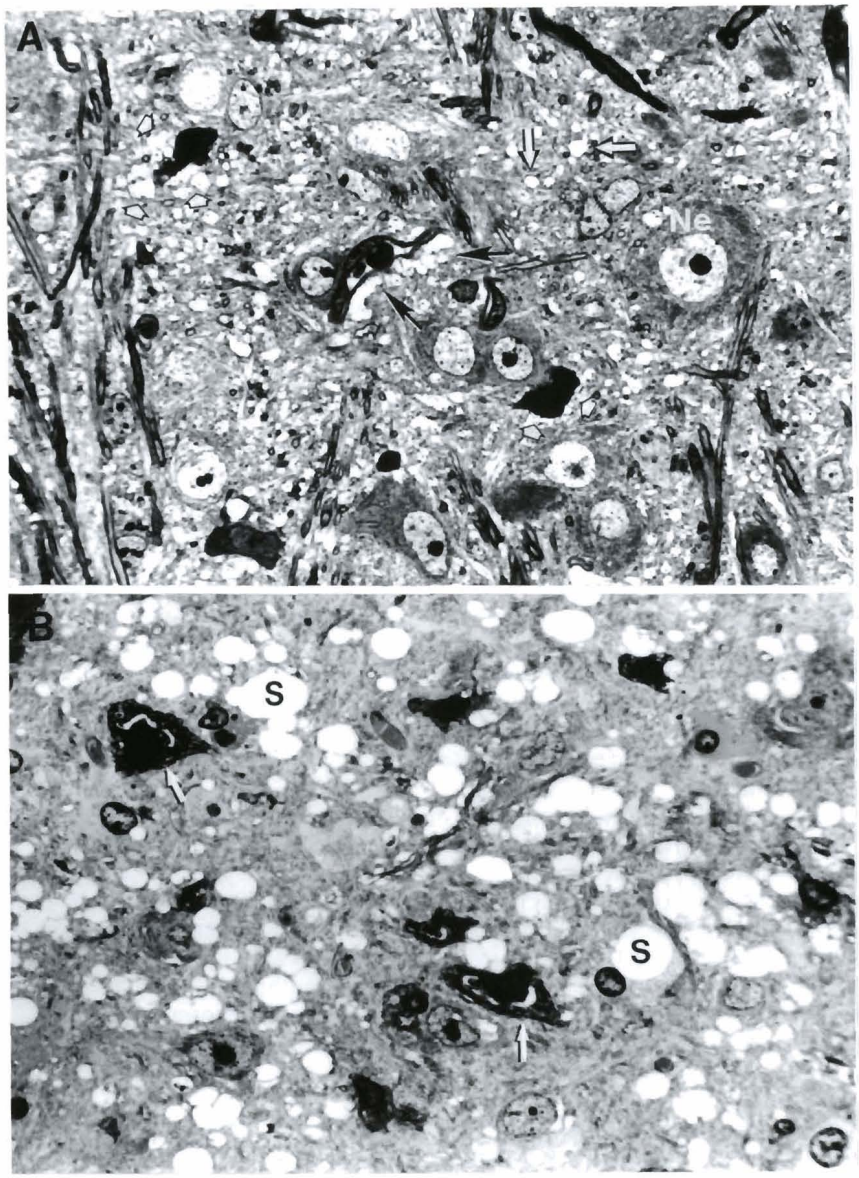


**Fig. 1. A.** Histologic section of a brain stem of a *ts*1 MoMuLV-infected FVB/N mouse killed at 12 days post-inoculation showing mild spongiform changes in the perivascular (black arrow) and perineuronal (short white arrow) locations and within the neuropil (thin white arrow). Notice also degenerating neuronal cells (dark cells with perineuronal spongiform changes) and a healthy neuron (Ne). Epoxy section (1 μm) toluidine blue. x 1,200 (from Gonzales-Scarano et al., 1995, with permission). **B.** Histologic section of lumbar spinal cord (ventral horn region) from a *ts*1 MoMuLV-infected FVB/N mouse killed at 30 days post-inoculation. At this stage of disease progression the lesions are characterized by severe neuropil spongiform changes (S) and apoptotic neurons (arrows) showing cellular and nuclear condensation and intractyoplasmic vacuoles. Epoxy section (1 μm) toludine blue. x 1,200 (from Gonzales-Scarano et al., 1995, with permission).

lesions may vary, lesions appear mainly in the brain stem and spinal cord (particularly in the anterior lateral horns) and to a lesser extent in the cerebellum (Table 1). Lesions in the lumbar region are generally more severe than those in the cervical region of the spinal cord.

The clinical features seen in this disease point to selective or regional loss of neuronal cells. This is reflected in the well-defined topography of spongiform lesions in the CNS (Wong, 1990; Jolicoeur et al., 1992; Stoica et al., 1993). The exact reason for this selective distribution is not known but it has been attributed to the influence of the sequences within the long terminal repeat (LTR) and *env* gene of the virus (Portis, 1990; Lynch et al., 1991; Jolicoueur et al., 1992; Wong and Yuen, 1992).

Within specific CNS regions affected there appear to be substantial differences between models in the extent of the lesions in the grey and white matters. In CasBrE- and $FrCas^E$-induced diseases the lesions are more concentrated in the grey matter than in the white matter (Table 1). In *ts*1 MoMuLV infections particularly in FVB/N mice (Wong et al., 1991), both grey and white matter are equally involved (Stoica et al., 1993). The lesion in the grey matter is primarily confined to the region where neurons are found, but the nature of the white-matter lesions has not, thus far, been well described. The extent of demyelination also varies among viruses or with the same virus in different strains of mice.

Although gliosis is present in most models, the role of gliosis in neuropathogenesis is unclear. In CasBrE- or *ts*1 MoMuLV-infected mice the appearance of spongiform lesions preceeds that of gliosis, indicating that gliosis is secondary to neural pathology (Nagra et al., 1993; Stoica et al., 1993). Although the development of spongiform lesions in *ts*1 MoMuLV-infected mice is faster in general than it is in CasBrE-infected mice, the gliosis is milder in the *ts*1-MoMuLV-infected mice (Nagra et al., 1993). Since gliosis is a response to CNS injury that takes time to develop, it is not surprising that gliosis is less frequent and is milder in *ts*1-Mo-MuLV-infected than in CasBrE-infected mice. That gliosis is correlated with a slower disease progression is demonstrated in a recent study by Czub et al. (1994) which shows that gliosis also occurs in $FrCas^E$-infected mice when a vary low dose of the virus is used to slow down the rate of disease. Thus, the lack of gliosis in $FrCas^E$-infected mice reported previously (Lynch et al., 1991) is most likely due to the acute nature of the disease, which occurs within 14 days postinfection.

### Cell types in the CNS infected by the viruses

To understand the pathogenic mechanisms of spongiform lesions and neuronal cell death induced by MuLV infection, it is crucial not only to identify the CNS cell types infected by the virus, but also to evaluate critically whether the cell types infected are directly or indirectly involved in neuronal degeneration. This, however, is a very challenging task because of the extreme complexity of the cell types and their respective subtypes that compose the CNS tissue and the technical

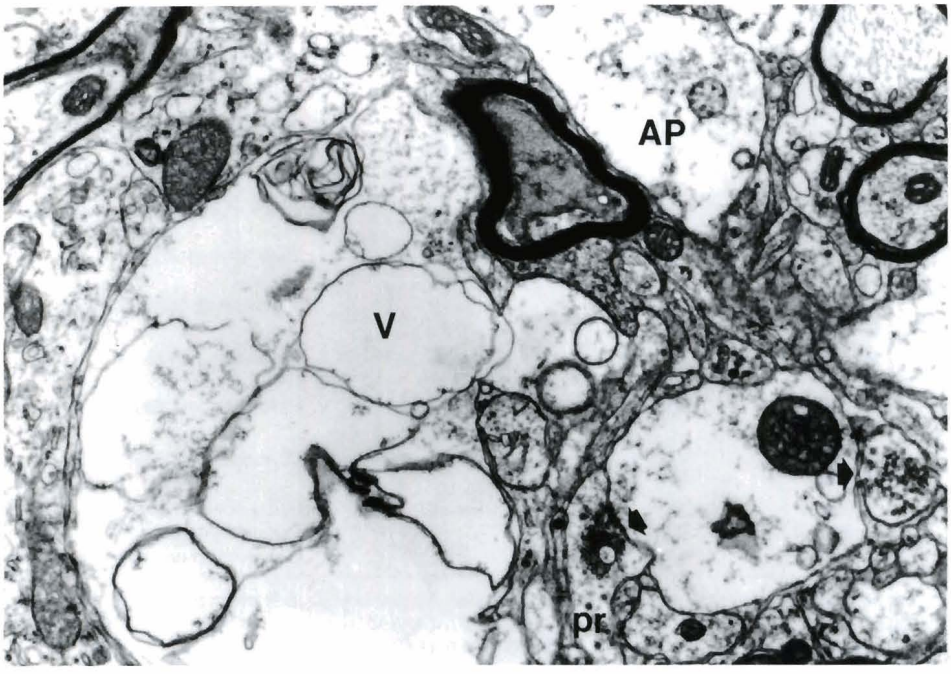


**Fig. 2.** Electron-microscopic examination of vacuolar changes in the brain stem medulla of a *ts*1 MoMuLV-infected mouse 15 days post-inoculation. There was vacuolization associated with the postsynaptic endplates (arrows). Presynaptic (pr) vesicles were still abundant despite postsynaptic degeneration. Also note the membrane-bound vacuoles (V) within the neuropil and a swollen astrocytic foot process (AP). x 16,000. (from Stoica et al., 1993, with permission).

problems is correctly identifying which cell types are infected. A number of investigators have attempted to resolve these difficulties using a variety of techniques, which include immunohistochemistry (Brooks et al., 1979; Oldstone et al., 1980, 1983; Hoffman et al., 1988; Morey and Wiley, 1990; Sharpe et al., 1990; Baszler and Zachary, 1991; Stoica et al., 1993), confocal microscopy (Nagra et al., 1993); electron microscopy (EM) (Andrews and Gardner, 1974; Oldstone et al., 1977, 1980, 1983; Swarz et al., 1981; Bilello et al., 1986; Zachary et al., 1986; Pitts et al., 1987; Baszler and Zachary, 1990; Stoica et al., 1993), and in situ hybridization (Kay et al., 1991; Lynch et al., 1991; Gravel et al., 1993). Despite these efforts, much debate remains as to which cell types in the CNS are truly infected by which neuropathogenic MuLVs in which

**Table 1.** Comparison of murine retroviruses that cause spongiform neurodegeneration.

| VIRUS | DERIVATION OF VIRUS | MOUSE STRAIN | INCIDENCE/ LATENCY | SPONGIFORM DISTRIBUTION | CELLS INFECTED | REFERENCE |
|---|---|---|---|---|---|---|
| CasBrE | Original wild Isolate | LC feral | 10% by 12 months | Lower SC | En, Ne, Gl | Gardner et al., 1973 |
| CasBrE | LC Isolate | NIH Swiss | 100% by 5 months | SC, BS | En, Gl, Ne | Andrew and Gardner, 1974 |
| CasBrE | LC Isolate | NIH Swiss | 90% by 4-5 weeks | SC, BS, CB GM | En, Pr, As,Ne | Brooks et al., 1980 Swarz et al., 1981 |
| CasBrE | LC Isolate | SWR/J | 100% by 6 months | SC, BS, GM | En, Ol, Ne | Oldstone et al., 1977, 1980, 1983 |
| NE-8 | Molecular clone of CasBrE | SWR/J | 100% 5months; 3 weeks (IIU) | SC, BS, CB | En, AS, Ne | Sharpe et al., 1990 |
| NE-8 | Molecular clone of CasBrE | SWR/J C3H | 60% by 24 weeks | SC, BS, CB,GM | En, Ol, Ne | Morey and Wiley, 1990 |
| NE-8 | Molecular clone of CasBrE | C3H | 90% by 16 weeks | SC, BS, CB, GM | En, Ol Ne | Nagra et al., 1992 |
| NE-8 | Molecular clone of CasBrE | CFW | >90% by 10 weeks | SC, BS, GM | En, Ep Mi, As, Ol, ? Ne | Nagra et al., 1993 Jolicoeur et al., 1991 |
| CasBrM | LC Isolate | NFS/N | 100% by 14 weeks | SC, BS, CB GM, WM | En, As, ?Ne | Pitts et al., 1987 Hoffman et al., 1991 |
| 15-1 | Molecular clone of CasBrM | IRW | 8% > 24 Weeks 100% 3-4 weeks (IIU) | SC,BS,CB GM, WM | not examined | Portis et al., 1990 Lynch and Portis 1993 |
| FrCas$^E$ | Chimeric of 15-1 and FrMuLV | CFW IRW | 100% by 2.5 weeks | SC, BS, CB,GM | En, Pr ?Ol, Mi Ne | Lynch et al., 1991 |
| *ts*1 | MoMuLV | FVB/N | 100% 3-5 weeks | BS, SC, CB TH, GM, WM | En, Pr, Ep, As, ol, Mi, ?Ne | Stoica et al., 1993 |
| *ts*1 | MoMuLV | BALB/c | >90% by 7-8 weeks | SC, BS, CB GM, WM | En, Ol, Mi, Ne | Nagra et al., 1993 |
| *ts*1 | MoMuLV | CFW/D | >90% by 5-7 weeks | SC, BS, CB GM | En, Gl, ?Ne | Zachary et al., 1986 |
| *ts*1 | MoMuLV | CFW/D | >90% by 7 weeks | SC, BS, CB, GM | En, Pr, Mi | Baszler and Zachary, 1990 |
| *ts* MoBA-1 | MoMuLV | NFS | >90% 10-20 weeks | Upper SC, BS, CB | En, Gl | Bilello et al., 1986 Pitts et al., 1987 |
| PCV-211 | Fr-MuLV | NFS/N | >90% by 4 weeks | SC, BS, CB | En | Hoffman et al., 1992 |
| PCV-211 (cloned 3d) | Fr-MuLV | F344 rat | >90% by 4 weeks | SC, BS, CB GM, WM | En | Masuda et al., 1992 |
| F-MCF-98D | Fr-MuLV MCF | IRW | >50% by 6 months | CB, WM, GM | En, Gl, | Buller et al., 1990 Portis and Chesebro (personal communication) |

All mice were infected as newborns (within 48 hours after birth) except when specified otherwise. As: astrocytes; BS: brain stem; CB: cerebellum; En: endothelial cells: Ep: ependymal cells; Gl: glia; GM: grey matter; IIU: inoculated in utero; LC: Lake Casitas; Mi: microglial cells; Ne: neurons; Ol: oligodendrocytes; Pr: pericytes; TH: thalamus; SC: spinal cord; WM: white matter. Modified from Gonzalez-Scarano et al., 1995, with permission.

mouse strains and the role each infected cell type plays in neuropathogenesis. These uncertainties probably have arisen because of differences in the virus and mouse strains employed by the different groups of investigators. The following problems have very likely contributed to the difficulties:

*1. Problems in identifying the cell types of the CNS*

a. The lack of specificity of the different cell markers used could present a problem. It is recognized that markers for neurons and glial cells are not completely reliable. For example, in most cases identification of neuronal cells is based on the presence of neurofilaments, which are abundant only in the soma of large neurons (Barnstable, 1992). The identification of astrocytes by the presence of glial fibrillary acidic protein (GFAP) using immunostaining is also problematic because astrocytes are heterogeneous with respect to the expression of GFAP (Shinoda et al., 1989; Landry et al., 1990; Mirsky and Jessen, 1991; Langan and Slater, 1992; Riol et al., 1992). In addition, whereas the presence of lectins in astrocytes may be specific under some pathologic conditions (Suzuki et al., 1988; Hulette et al., 1992), the lectins may also bind to other glial cells (Hulette et al., 1992). In addition, in mice there are no known unique phenotypic markers for resting microglia (Benveniste, 1992). Because specific markers for different cell types present in the CNS are lacking, other criteria such as morphologic structure and location have also been used. Identifying cells on the basis of morphology, however, has not been completely satisfactory either because of the morphologic appearance of some cell types varies and a large number of infected cells may not fit the description of known CNS cell types, particularly during the late stages of disease in which cellular morphology is distorted by pathologic changes. As a result a large number of virus-infected cells have been labeled as unclassifiable by some investigators (Morey and Wiley, 1990; Nagra et al., 1993).

b. Another problem in identifying neural cells infected with a virus is that neural cells such as neurons, oligodendrocytes, and astrocytes may undergo apoptosis and be rapidly removed by phagocytes in the brain, without inducing an inflammatory response (Raff et al., 1993). This rapid removal of the virus-infected cells by phagocytes prevents their identification when disease tissues are examined. To add to this problem, the virus-infected cells phagocytized by microglia may give the impression that the phagocytes are actually replicating the virus. Therefore, the presence of intracellular viral RNA and proteins or particles may not necessarily indicate that these cells are replicating the virus.

*2. Problems in identifying virus in infected cells*

a. The specificity and sensitivity of the reagents and techniques used to detect specific viral components in the cell can also be problematic. For example, although EM so far is one of the best means of observing virus particles in infected cells, EM is unfortunately not very sensitive. Studies relying on immunohistochemical techniques to detect the presence of virus are also problematic because retroviruses are generally only weakly immunogenic, and it is difficult to generate sensitive immunohistochemical probes for the viral antigens (Wiley and Gardner, 1993). This is particularly true when monoclonal antibodies are used. Because of the weak labeling of viral antigens with monoclonal antibodies, colocalization of virus antigens with cell markers by double labeling often results in the former being masked by the latter. The problem is compounded because the low expression of viral antigens in the quiescent cells in the CNS renders a large proportion of infected cells not readily detectable. Confocal microscopic analysis, which can «optically section» thick samples, can increase the probability of detecting viral antigens and cell markers (Murray, 1992), but so far, this technique has not been widely used.

In situ hybridization offers another monospecific means of detecting viral transcripts in infected cells. However, in situ hybridization is not always sensitive enough to pick up low levels of virus expression. A case in point is that of studies with transgenic mice that harbor the viral envelope gene of the CasBrE virus (Kay et al, 1993). Although a milder form of the spongiform lesions was observed by histopathologic studies and viral envelope mRNA expression, was detected by RNase protection assay in the CNS of these transgenic mice, in situ hybridization analysis failed to detect any viral envelope mRNA in these mice (Kay et al., 1993). In situ polymerase chain reaction (PCR) may provide the specificity and sensitivity needed, but this technique has not yet been used in murine systems, although recently Nuovo et al. (1994) using this method, detected HIV RNA in the CNS of patients with AIDS dementia.

b. The nature of the virus infection in the CNS may also contribute to the difficulty of identifying which cell types are infected. The adult CNS contains a large population of quiescent cells. Most of these are post-mitotic neurons, oligodendrocytes, astrocytes, and micro-glial cells. Replication of a retrovirus in these cells may be abortive, restrictive, or transient. For example, since incomplete reverse-transcription of the MuLV genome in quiescent non-dividing cells was recently reported (Zack et al., 1992), virus expression in these cells may not be recognized by either antibodies or viral probes.

c. A potential problem in identifying the target cell infected by the injected virus could be caused by the inability of the reagents used to distinguish between exogenous and endogenous MuLV components. Fortunately, so far no evidence of expression of endogenous virus in the CNS of any of the MuLV-induced neurologic

diseases has been established (Wiley and Gardner, 1993). Given the ubiquitous presence of endogenous murine retroviruses, however it is difficult to exclude conclusively their involvement in any of the diseases induced by MuLV. The pros and cons of the role of endogenous viruses in MuLV-induced neuronal degeneration will be discussed below.

Despite the problems presented above, most investigators in this field now agree that CNS capillary endothelial cells and probably also pericytes can be productively infected (Table 1). In a recent study, ependymal cells in the ventricular area of the fourth-ventricle choroid plexus have also been reported to be productively infected (Stoica et al., 1993). Some astrocytes as well as microglial cells can also be infected, and oligodendrocytes are also targets of the virus. Whether neurons can be infected remains the most controversial issue. Some groups have reported that neurons, with the exception of cerebellar granule cells (Lynch et al., 1991), Purkinje cells (Stoica et al., 1993), and interneurons (Jolicoeur et al., 1992), do not express viral antigen. Several studies by other researchers, however have detected viral antigen or virus particles in neurons in disease areas, particularly during late stages of the disease. An attempt to summarize the studies on the cell types of the CNS infected by different MuLVs and their possible role in the pathogenesis of neuro-degenerative disease is presented below.

### Endothelial cells and pericytes

Capillary endothelial cells, and probably also pericytes, are the CNS cell types most conspicuously infected by all the neuropathogenic MuLVs examined (Table 1). Virus-infected endothelial cells are readily identifiable because of their location, i.e., they line the internal surfaces of blood vessels. In addition, vascular endothelial cells appear to be the cells in the CNS infected earliest by the neuropathogenic MuLVs. In most cases viral replication in these cells can be detected within 1 to 2 weeks after injecting neonates (Lynch et al., 1991; Swarz et al., 1981; Stoica et al., 1993). At this age the mice are actively growing, as are the endothelial cells in the CNS. As a consequence, CNS endothelial cells are highly supportive of retroviral replication. That dividing cells play an important role in the replication of retroviruses in the CNS has been demonstrated in chickens by studying an in ovo infection with a neuro-tropic avian retrovirus. This study showed that the highest levels of virus replication were localized in vascular endothelium, the choroid plexus, and the granular layer of the cerebellum (Ewert et al., 1990). All these areas contain a high proportion of mitotic cells.

In spite of the observation that endothelial cells are highly supportive of neuropathogenic MuLV replication, the exact role that viral replication in these cells plays in neuronal cell death remains unclear. In most instances, viral replication in endothelial cells does not produce any observable changes in the gross morphology of these cells. Only one report observed that replication of Cas-Br-M and *ts*MoBA-1 in capillary endothelial cells was accompanied by marked changes in the ultrastructure of the basal lamina (Pitts et al., 1987). Pitts and coworkers suggested that the primary mechanism responsible for vascular degeneration may involve basal lamina disruption by the budding virus and that this may in turn disturb the homeostasis of the surrounding parenchyma. This homeostatic imbalance may initiate or augment the spongiform changes in the neuropil and neuronal perikarya.

Jolicoeur et al. (1992) reported that CNS endothelial cells are not equally infected although they are equally exposed to the CasBrE virions that circulate in the bloodstream. They suggested that endothelial cells in various CNS anatomic sites may have distinct properties and may interact differently with the virus.

Other researches, however, have observed that vascular endothelial cell infection is widespread in the CNS and its distribution is not restricted to the location of spongiform lesions (Lynch et al., 1991; Stoica et al., 1993). In fact, a high proportion of endothelial cells is infected in some nondiseased regions, than in diseased regions (Gravel et al., 1993). In view of these findings, one cannot conclude that the mechanism responsible for spongiform lesions in specific regions of the CNS is mainly the disruption of infected endothelial cells. The high level of replication of the MuLVs in the CNS vascular endothelial cells may, instead, simply provide a means for viral entry into the CNS.

In the case of PVC-211 infection in rats or mice, endothelial cells appear to be the only cell type in the CNS in which the virus replicates. In this case, however no direct effect was observed on gross morphology or disfunction of the CNS capillary endothelial cells (Hoffmann et al., 1992; Masuda et al., 1992). This observation led to the suggestion of an indirect neuro-pathogenic mechanism involving neurotoxin released by the infected endothelial cells.

### Ependymal cells

A recent study identified viral antigens and virions within the ependymal cells of the fourth ventricle and within the ventricular and central canal lumen during the early stages of disease in *ts*1 MoMulV-infected FVB/N mice (Stoica et al., 1993). This observation suggests that the infection in ependymal cells may allow the virus to gain access to the CNS via the cerebrospinal fluid, thus agreeing with a report that the spread of HIV-1 to the brain is an early event during infection and may occur via the cerebrospinal fluid (Chiodi et al., 1992).

### Astrocytes

Several reports have shown that astrocytes are infected by CasBrE (Sharpe et al., 1990; Jolicoeur et al.,

1991; Swarz et al., 1981), Cas-Br-M (Hoffman et al., 1991), and *ts*1 MoMuLV (Stoica et al., 1993). Astrocytes have also been observed to be infected by LP-BM5 MuLV (Sei et al., 1992). Several other investigators, however have failed to detect viral infection of astrocytes by CasBrE (Morey and Wiley, 1990; Nagra et al., 1993), $FrCas^E$ (Lynch et al., 1991), and *ts*1 MoMuLV (Bazler and Zachary, 1990, 1991; Nagra et al., 1993). The discrepancy in these findings could result from the following:

1. So far, GFAP immunostaining is the technique used most often to identify astrocytes. Astrocytes are heterogeneous in function and in expression of proteins, including GFAP. For example, GFAP has been shown to be down-regulated in the adult mouse brain, and GFAP staining is minimal despite the abundance of astrocytes (Landry et al., 1990). Many grey-matter astrocytes label poorly with GFAP antibodies (Mirsky and Jessen, 1991). Several reports also indicate that virus infection of astrocytes could result in a decrease in GFAP in these cells (Itoyama et al., 1991; Rinaman et al., 1993). Pulliam et al. (1993) have also reported that the gp120 envelope protein of HIV can cause decreased expression of GFAP in astrocytes. Therefore, without some sort of activation, GFAP immunostaining may not be sensitive enough to identify certain astrocytes or subtypes of astrocytes, especially when attempts are made to colocalize expression of viral antigens and GFAP.

2. Activated astrocytes in gliotic areas may not necessarily be the astrocytes infected by the virus, but rather they may be reactive astrocytes that responded to the tissue damage. However, astrocytes infected by MuLV may have become reactive without expressing an increased level of GFAP. These notions have been supported by several observations. Hoffman et al. (1991) reported that although astrocytes were infected by CasBrM virus in the region exhibiting spongiform lesions, the majority of astrocytes in gliotic areas did not express MuLV gp70. Jolicoeur et al. (1991) reported that in CasBrE-infected mice virtually all infected cells in areas exhibiting spongiform lesions appeared to be nonneuronal cells and most seemed to be astrocytes; however, double-labeling with virus-specific in situ hybridization together with anti-GFAP showed that only about 5% of these cells were unambiguously identified as astrocytes. By using double-label immunohistochemistry, Nagra et al. (1992) found no colocalization of GFAP and viral antigens, despite pronounced astrogliosis in CasBrE-infected SWR/J mice. A similar double-staining study by Sharpe et al. (1990) revealed colocalization of GFAP and viral antigens in only a small number of astrocytes. In FVB/N mice infected with *ts*1 MoMuLV double labeling revealed positive viral envelope antigen as well as GFAP immunostaining, and virus particles, budding from the granular endoplasmic reticulum (ER) in cells bearing GFAP, could be detected by EM although the latter events is not as frequently observed (Stoica et al., 1993). Astroglial response in the spongiform neurodegeneration induced by PVC-211 MuLV in mice or rats in the absence of viral antigens in these cells was also observed (Masuda et al., 1992).

3. Some viral infection of astrocytes appears to be restrictive, i.e., infectious viruses are produced at a low level. This could result from active suppression of viral replication by astroglial factors or within the astrocytes by lack of factors for efficient transport, processing, and assembly of the viral components. Inefficient transport and processing of the retroviral envelope protein precursor in astrocytes has been shown recently in a primary culture of astrocytes infected with *ts*1 MoMuLV (Shikova et al., 1993). In addition, cell-type-specific modification of retroviral envelope glycoprotein has been observed in feline immunodeficiency virus-infected astrocytes in vitro (Poss et al., 1992).

4. The detectability of viral expression in astrocytes could also be influenced by the virus and the strains of mice used. For example, in *ts*1 MoMuLV-infected mice, viral expression in astrocytes was more readily detectable in the FVB/N strain (Stoica et al., 1993), than in the BALB/c strain (Nagra et al., 1993). A comparison of infectious virus production from *ts*1 MoMuLV-infected primary astrocyte cultures of FVB/N mice and that from *ts*1 MoMuLV-infected primary astrocyte cultures of BALB/C mice showed a ten-fold higher virus titer in the former than the latter (Wong, unpublished data). Thus, variations in the detectability of virus expression in astrocytes could be due to the level of virus replication in the astrocytes of different mouse strains.

Other compelling evidence suggests that astrocytes can be infected by retroviruses and that these virus-infected astrocytes may play critical roles in neuronal cell degeneration. In vivo studies indicate that the perivascular astrocyte foot processes are the first structures of glial cells to show vacuolar changes in Cas-BrE (Swarz et al., 1981; Nagra et al., 1992), Cas-BR-M (Hoffman et al., 1991); and *ts*1 MoMuLV (Stoica et al., 1993)-infected mice. As the disease progresses, vacuolization within the astrocytic cell body also occurs. Furthermore, mitochondrial dissolution and vesicular enlargement of the Golgi and ER before cellular disintegration have also been observed in astrocytes (Stoica et al., 1993). This together with positive viral-antigen immunostaining and the observation of virus budding from granular ER in astrocytes (Stoica et al., 1993), strongly subtantiates at least in the case of *ts*1 MoMuLV, that the cytopathic effect observed in astrocytes could result from viral infection of these cells.

Several reports also indicate that HIV can directly infect and replicate in cultured astrocytes (Cheng-Mayer et al., 1987; Chiodi et al., 1987; Christofonis et al., 1987; Dewhurst et al., 1987; Brack-Werner et al., 1992; for review see Gonzalez-Scarano et al., 1995). Some of these reports also describe mild morphologic changes (Dewhurst et al., 1987) or temporary cell-growth retardation in HIV-infected astrocyte cultures (Chiodi et al., 1987; Brack-Werner et al., 1992).

In vivo astroglial expression of HIV-1 gp120 in transgenic mice induces neuropathologic alterations that closely resemble changes seen in brains of patients infected with HIV-1 (Toggas et al., 1994), indicating that virus products expressed in astrocytes can induce neuronal pathology. More recently, Nuovo et al. (1994), using an in situ PCR technique, have demonstrated the presence of HIV RNA in astrocytes, microglial cells, and neurons of patients with AIDS dementia.

Since astrocytes compose a substantial proportion of the cell population in the CNS and since they also function as neuronal cell helpers in maintaining environmental homeostasis surrounding the neurons (Benveniste, 1992), impairment of astrocytes by viral infection may affect the wellbeing and survival of neurons. In addition, astrocytes also exert a protective role on oligodendrocytes and interact with microglia to produce cytokines (Mucke and Eddleston, 1993), which could also affect the neurons.

### Microglial cells

Microglial cells are antoher cell type in the CNS reported to express MuLV in mice infected with various strains of neuropathogenic MuLVs. However, whether microglia are invariably infected by these viruses is not completely non-controversial since several investigators (Oldstone et al., 1980, 1983; Morey and Wiley, 1990; Sharpe et al., 1990; Swarz et al., 1981; Nagra et al., 1992, 1993) have been unable to detect MuLV antigens in the microglia of CasBrE-virus infected mice. Why viral antigen expression was not detected in these studies remains unclear.

Interestingly, recent studies have shown that CasBrE virus-infected microglial cells frequently appeared in the CNS as clumps or micronodules (Gravel et al., 1993) and that giant multinucleated microglial cells were occasionally present within the microglial nodules of *ts*1 MoMuLV-infected FBV/N mice (Stoica et al., 1993). Giant multinucleated microglial cells, however, have not been reported previously in the CNS of any mice infected with neuropathogenic MuLVs. Whether these observations parallel that of the HIV-induced syncytia formation of microglia in AIDS-related neuropathy is unclear and warrants further investigation.

Some investigators have indicated that microglial cells are the major infected-cell population in the CNS and suggested that this cell type plays the most crucial role in neuropathogenesis (Baszler and Zachary, 1990; Gravel et al., 1993). However, as mentioned above, the readily detectable presence of viral nucleic acid and antigens within microglia does not necessarily mean that these cells are productively infected by the virus. Since the major known function of microglia is phagocytosis of impaired cells, cells debris, and foreign agents (Perry and Gordin, 1988; Banati et al., 1993); the presence of viral components within these cells could be the result of phagocytosis of viral-infected cells and virus particles in the diseased region. The increased concentration of microglia with detectable viral components in the diseased region could reflect the natural response of these cells to the damaged tissue.

The ability of microglia to release a large number of infectious viruses to the extracellular space has not been documented in vivo or in vitro. Although virions can be detected ultrastructurally within the microglial cytoplasm of *ts*1 MoMuLV-infected FVB/N mice, they are not abundant, and evidence of budding viral particles at the cell membrane has not been found (Stoica et al., 1993). In $FrCas^E$ virus-infected microglial cells, virus particles are predominantly observed intracellularly, and little infectivity is detectable in the supernatant (Lynch et al., 1994). That microglial cells were not producing a large amount of infectious HIV was also observed recently in an ex vivo study by Brinkmann and coworkers (1992). These investigators showed by infectivity assay that microglial cells isolated from the brain tissue of a child dying of AIDS produced only a small amount of infectious virus, even after a prolonged period of culture.

In the most rapid neurologic disease induced by the $FrCas^E$ virus, where the disease ran its entire course in 21 days, no activation of microglia by viral infection was observed (Lynch et al., 1991), and the detection of viral antigens in microglial cells lagged behind disease sympthoms (Czub et al., 1994). In fact, a significant amount of viral antigens was only detected in the microglia at late stages of the disease.

The above findings, taken together, argue that the presence of virus in microglia may be a secondary event in the pathogenic process. As pointed out above, replication of virus in microglial cells is generally slow, and only a small amount of virus is released into the extracellular space. Since microglia constitute only approximately 10% of the total glial cell population (Benveniste, 1992), it is unlikely that microglia alone can generate widespread and extensive neuronal death within specific regions of the CNS. It may be more likely that microglial cells in concert with astrocytes play key roles in this processes. The significance of microglial infection in the pathogenesis of neurodegeneration remains to be clarified and will be further discussed later.

### Oligodendrocytes

Owing to the difficulty in correctly identifying oligodendrocytes in the CNS, infection of these cells has been hard to prove unambiguously. Several groups, however, have reported that oligodendrocytes are viral targets (Table 1). In a recent study (Stoica et al., 1993), budding C-type particles were observed at the cell surface of oligodendroglial cells of FBV/N mice on the 20th day after infection with *ts*1 MoMuLV. Viral replication in these cells was primarily periaxonal and was associated with axonal dissolution, vacuolization, myelin splitting and collapse. Abundant mature virions were also present intracytoplasmically at a later stage of the disease.

Whether oligodendrocytes actively participate in events leading to neuronal degeneration is unclear, and further studies are necessary.

### Neuronal cells

Despite the fact that motor neuronal loss is the most prominant feature of the spongiform degenerative diseases, replication of MuLV in these cells is generally not readily detectable. Lynch et al. (1991) found no evidence that neurons located in sites of spongiform lesions were infected by either the $FrCas^E$ or the $CasBr^E$ virus, despite using highly specific probes in a variety of techniques. Kay et al. (1991), using a similar in situ probe to that used by Lynch et al. (1991), also failed to detect CasBrE infection of motor neurons in regions undergoing neurodegeneration, although virus was detected in glia and endothelia. Immunohistochemical studies of the distribution of *ts*1 MoMuLV in the infected CNS also failed to detect viral proteins in neurons in those regions undergoing spongiform degeneration during the early stages of the disease (Zachary et al., 1986; Baszler and Zachary, 1990, 1991; Stoica et al., 1993). Contrary to these observations, other investigators using immunohistochemistry, have readily detected viral proteins in neurons in the disease region of CasBrE- (Morey and Wiley, 1990; Sharpe et al., 1990; Nagra et al., 1993); *ts*1 MoMuLV- (Nagra et al., 1993); and WT MoMuLV-infected mice (Sharpe et al., 1990). These studies, however used polyclonal anti-whole virus sera. Whether these antibodies were capable of distinguishing the proteins of the exogenous virus from those of the endogenous virus remains unclear. Nevertheless as pointed out above, the fact that polyclonal antibodies are more sensitive, albeit less specific, than monoclonal antibodies, may explain why studies with polyclonal antibodies can more readily detect viral expression in neurons than can monoclonal antibodies. Using EM, several investigators (Andrews and Gardner, 1974; Oldstone et al., 1977, 1980, 1983; Swarz et al., 1981) have identified virus particles in neuronal cells of CasBrE-infected NIH swiss and SWR/J mice, but generally late in the disease and not often in regions undergoing degeneration. These findings prompted some investigators to suggest that the presence of viral particles in neurons may be caused by the activation of endogenous virus in response to stress as a result of spongiform degeneration.

The role of the inoculated MuLV versus activated endogenous MuLV or recombinant virus, however, has been controversial. Although, CasBrE/CasBrM-induced spongiform neurodegenerative disease, viral recombination and production of dual tropic MuLV is somewhat evident in the spleen (Hoffman et al., 1981; Oldstone et al., 1983) it has not been detected in the CNS (Hoffman et al., 1981). The argument that endogenous virus may be activated in the CNS as a result of stress caused by spongiform neurodegeneration is not consistent with the findings that neither endogenous MuLV proteins nor particles were detected in the CNS of mice suffering from similar spongiform neurodegeneration induced by the scrapie agent or by the transmissible agent associated with gray-tremor mutation gt/gt (Hoffman et al., 1982, 1987). The hypothesis that endogenous MuLV could be activated by integration with exogenous MuLV is also unlikely, since most of the neurons are quiescent nondividing cells, and integration of retrovirus requires mitosis (Roe et al., 1993).

In conclusion, the above observations indicate that an endogenous virus in the CNS is not likely to be activated by stress or by injected MuLVs. This conclusion is consistant with the notion that neurodegenerative disease is caused directly by the injected neuropathogenic MuLVs. The delayed appearance of virus-particle budding in neurons might be explained by the initiation of DNA repair in damaged neurons, allowing exogenous virus to be expressed from a latent state. The fact that dividing neurons, such as granule cells, in the cerebellar cortex, can support a high level of virus replication (Lynch et al., 1991) suggests that there is no neuron-specific restriction of retroviral infection at the receptor level. However, the late appearance of the virus particles observed in neurons may argue against their role in causing neuronal death.

Taken together, these studies indicate that all cell types in the CNS can be infected by one or more of the neuropathogenic MuLVs. Virus replication can be more readily detected in the endothelial cells and other actively mitotic cells in the CNS of young mice than in the quiescent cell types of neuroectodermal origin in older mice, where virus expression may not be readily detectable although the mice remain suceptible to virus infection. Although the presence of virus or viral components is more readily detectable in some instances, in microglial cells, this may not necessarily mean that these cells are productively infected. Astrocytes, being highly heterogeneous, may not be uniformly infectable or able to support viral replication. Oligodendrocytes are targets for some MuLVs, but the extent to which replication occurs and its effect on demyelination and neuronal degeneration remains unclear. Some neurons, such as interneurons and granules are supportive of virus replication, but others, such as motor neurons, are either nonsupportive or restrictive of viral replication. Because, in most instances, MuLV replication in motor neurons is not apparent, neurodegeneration more likely results from indirect mechanisms of neuronal cell killing by the virus.

### Pathogenesis of MuLV-Induced Neurodegeneration

We propose now a model of MuLV neuropathogenesis that encompasses the findings obtained so far. After early replication in peripheral sites (primarily spleen and/or thymus), the virus spreads via the circulatory system to the endothelial and ependymal cells of the CNS (Fig. 3). After replication in these cells,

the virus gains access to the CNS by passing through the blood-brain barrier and the choroid plexus of the fourth ventricle. The virus could thus be disseminated within the CNS via the circulatory system and the cerebrospinal fluid.

Within the CNS parenchyma, microglial cells and astrocytes as well as oligodendrocytes becomes infected by the virus, although the degree of virus expression in these cells varies. Neurons display the most obvious cytopathic effect. Either these damaged neurons are rapidly removed by phagocytosis or they do not express detectable viral antigens or produce virus particles until the late stages of disease. The scarcity of detectable viral expression in neuronal cells suggests that neuronal cell killing is probably indirect, although a combination of direct and indirect mechanisms can not be excluded.

The virus may participate in the destruction of neurons by infection or by disrupting microglial cells, astroglial cells, and olygodendrocytes, which are required as helper cells to maintain the nearby neurons (Fig. 3). Several mechanisms of neuronal damage due to perturbation of helper cell function are possible. These include:

1. Activation of acute-phase response by astrocytes and microglial cells with resultant chronic over-production of neurotoxic growth-death signals and acute-phase antiproteases.

Several cytokines, in particular tumor necrosis factor alpha (TNFα) interleukine-1 beta (IL-1ß), and interleukine-6 (IL-6), have been implicated in neurologic disorders including viral disease (Frei et al., 1989) AIDS dementia (Gallo et al., 1989), and Alzheimer's disease (Bauer, 1991; Dickson et al., 1993).

Preliminary studies by Saha and Wong (unpublished data) from *ts*1 MoMuLV-infected mice also indicate

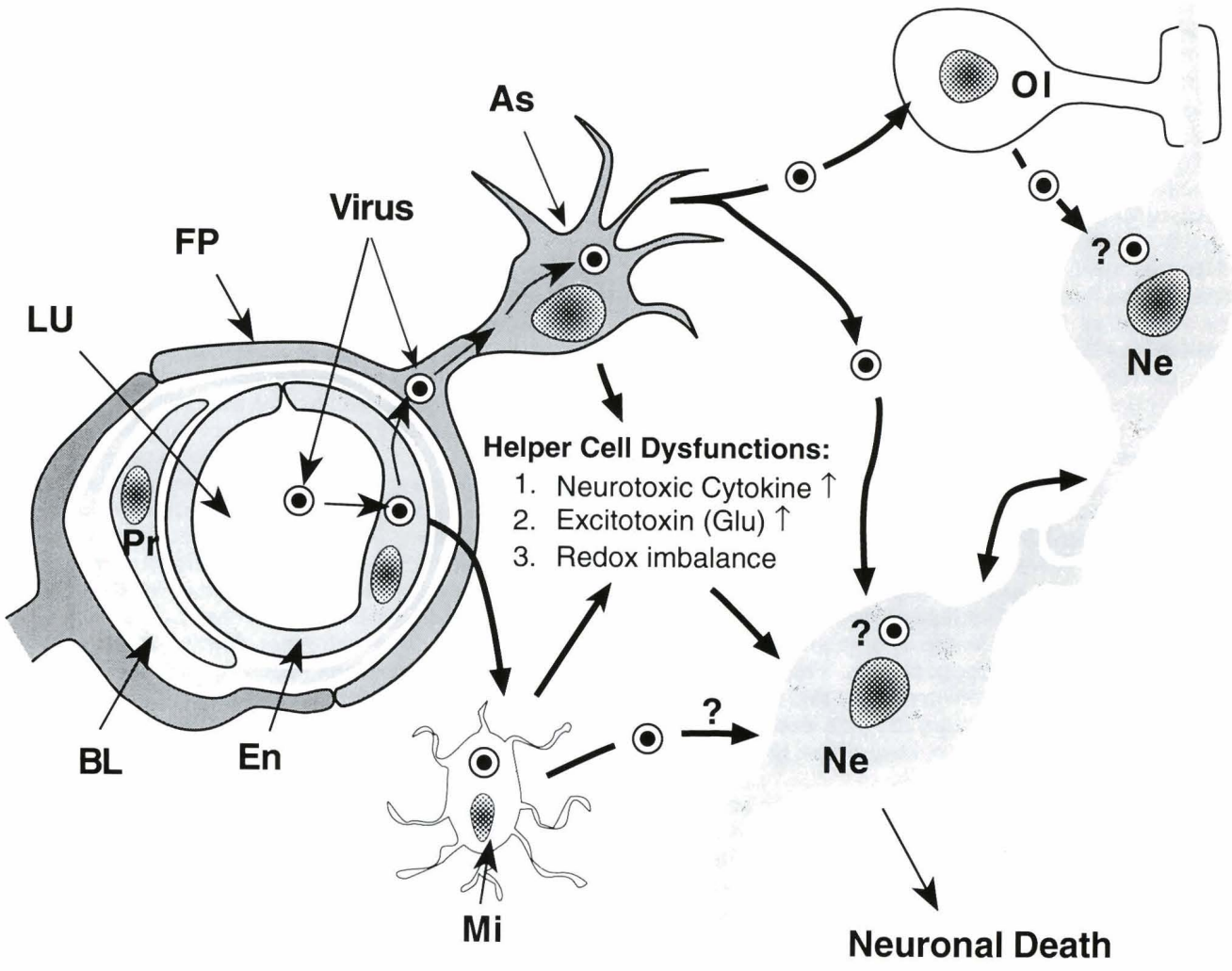


**Fig. 3.** Schematic presentation of the possible mechanisms of MuLV-induced neuronal damage. BL: basal lamina; FP: foot process; LU, lumen; En: endothelial cell; Pr: pericyte; As: astrocyte; Mi: microglial cell; Ne: neuron; OL: oligodendrocyte.

upregulation of TNFα and IL1ß in the CNS and serum. This observation suggests that these cytokines may be involved in the pathogenesis of MuLV-induced neurodegeneration. The source of these cytokines in the CNS, however, is not clear. Macrophages and microglial cells in the CNS have been shown to produce TNFα and IL1ß in AIDS dementia (Tyor et al., 1992). It has also been shown that stimulated astrocytes produce a TNFα-like substance (Epstein and Gendelman, 1993). In addition, overexpression of tumor growth factor beta (TGFß) has also been implicated as important in HIV neuropathogenesis (Wahl et al., 1991). Recently, transgenic mice whose astrocytes express high levels of IL-6 have been shown to develop a severe neurologic disease syndrome very similar to the neurologic disease induced by MuLVs, i.e., mice with reduced size, tremor, ataxia, hunched posture, and paralized hind limbs with extensive neuronal cell loss (Campbell et al., 1993). Acute-phase proteins, especially antichymotrypsin, also accumulate in the CNS of these IL-6 poisoned mice. These studies suggest that astrocytes and microglial cells in the CNS may be indirectly responsible for the CNS damage through the production of the above neuromodulators.

### *2. Failure of astrocytes to remove and metabolize the excess excitatory amino acids (EAAs)*

Recent findings suggest that HIV-1 associated neuronal damage can be mediated via EAAs (Epstein and Gendelman, 1993; Lipton and Rosenberg, 1994). Such EAA-mediated neurotoxicity may be further enhanced as injured neurons release glutamate (Pellegrini-Giampietro et al., 1990), damaging neighboring neurons, or by the failure of impaired astrocytes to adequately take up excessive glutamate. These observations suggest that EAA-associated neuronal damage is mediated in part via the activation by glutamate or other EAAs of one of the N-methyl-D-aspartate (NMDA) receptors, a mechanism that has been implicated in neuronal loss in a number of neuropathologic processes (Choi, 1991; Beal, 1992; Epstein and Gendelman, 1993; Lipton and Rosenberg, 1994). Ottersen and Storm-Mathisen (1985), using polyclonal antibodies have successfully localized the distribution of aspartate and glutamate in various regions of the brain. Using a similar approach, Stoica and Wong (unpublished data) have observed that, within the areas of *ts*1 MoMuLV-induced neurodegeneration, the intensity of the immunolabel for glutamate increases during the early phase of the disease before neuronal death. This suggests that glutamate may accumulate in the disease region in excessive amounts. The prolonged presence of this elevated interneuronal glutamate may trigger an oxidative stress cascade with resultant oxidative damage to DNA. This leads to loss of redox control, energy depletion, and cell death (Zhang et al., 1994).

The notion that EAAs such as glutamate are neurotoxic is substantiated by the ultrastructural evidence that the toxic process impinges focally on postsynaptic dendritic or somal membranes where excitatory synaptic receptors are located but does not disturb presynaptic axonal elements (Olney, 1983). The degenerative changes within the neuropil in CasBrE (Swarz et al., 1981; Nagra et al., 1992), FrCas$^E$ (Lynch et al., 1991), and *ts*1 MoMuLV (Stoica et al., 1993) infection have been shown to develop first at the postsynaptic sites (dendrite and soma) while preserving the axons and presynaptic terminals (Fig. 3). These findings concur with the concept that neuronal damage by these MuLVs could be mediated by the glutamate activation of NMDA receptors.

### *3. Excessive production or inefficient removal of neurotoxic oxidants and nitric oxide by the helper cells*

Excessive production or inefficient removal by astrocytes of nitric oxide (NO) and neurotoxic oxidants such as $H_2O_2$, $O_2^-$ can in the presence of $Fe^{2+}$, cause redox imbalance resulting in lipid oxidation and DNA destruction in the nearby neurons. In neurons, which, like T cells, are also probably dependent on their helper cells, i.e., astrocytes and microglial cells, for their supply of cysteine, a virus-induced cysteine and energy deficiency in the helper cells, with resultant elevation of extracellular glutamate concentration, could be the major factor responsible for oxidative-induced cell death (Hockenberg et al., 1993). Recent observations (Lynn and Wong, unpublished data) indicate that the virus-induced neuronal death in the *ts*1 MoMuLV murine model can be ameliorated in vivo to some extent by antioxidants, i.e., acetyl cysteine or melatonin, suggesting that redox imbalance and cysteine deficiency may be important contributors to neuronal cell death.

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