VIROLOGY 247, 127–136 (1998)
ARTICLE NO. VY989240

MINIREVIEW

Dissecting the Determinants of Neuropathogenesis of the Murine Oncornaviruses

John L. Portis*[1] and William P. Lynch†

*Laboratory of Persistent Viral Diseases, Rocky Mountain Laboratories, National Institute of Allergy and Infectious Diseases, Hamilton, Montana 59840; and †Department of Microbiology/Immunology, Northeastern Ohio University College of Medicine, Rootstown, Ohio 44272

Received May 15, 1998; accepted May 19, 1998

INTRODUCTION

The neurovirulence of most noncytolytic viruses that infect the brain is associated with the inflammatory responses they induce. Examples include coronaviruses (Lane and Buchmeier, 1997), Borna (Morimoto *et al.,* 1996), lymphocytic choriomeningitis virus (Buchmeier *et al.,* 1980) and Theiler's encephalomyelitis virus (Lipton and Jelachich, 1997). Infiltration of immune cells and disregulation of immune responses in the central nervous system are principally responsible for the clinical manifestations of these diseases. However, there are other agents that are neuropathogenic but that do not provoke inflammatory infiltrates. These include the transmissible spongiform encephalopathies ("prion" diseases) (DeArmond and Prusiner, 1998) and the neurodegenerative diseases caused by the murine oncornaviruses (Andrews and Gardner, 1974). The pathogenesis of these diseases appears to revolve purely around the interaction of the agent with cells intrinsic to the brain. In this review, we will discuss the principal viral and host determinants of neurovirulence induced by the oncornaviruses as well as the cellular players involved in the pathogenesis of these diseases.

Discovery and characterization of the Lake Casitas virus

In the early 1970s, Gardner and colleagues (1973) isolated an oncornavirus from the brains of wild mice at Lake Casitas in southern California. The virus, which was subsequently named Wild Mouse Virus or CasBrE, is mouse tropic (ecotropic) (Hartley and Rowe, 1976) and is endemic in this mouse population (Gardner *et al.,* 1976a,b). It is transmitted in external secretions (Gardner *et al.,* 1979; Portis *et al.,* 1987), primarily from mother to pup in the milk but also, among adults, by sexual intercourse and fighting. The provirus was not found to be present in the germline (Barbacid *et al.,* 1979). Mice inoculated as neonates develop a persistent and productive infection in many tissues of the body and exhibit a life-long viremia. Rarely (~10%) these mice develop a neurological disease manifested by tremor and paralysis with a long incubation period (preclinical period) ranging from several months to more than a year (Henderson *et al.,* 1974). The neuropathology is characterized by spongiform neurodegeneration primarily involving the motor system of the brain and spinal cord and associated with intense reactive gliosis and neuronal dropout (Andrews and Gardner, 1974; Brooks *et al.,* 1980). Infiltration of inflammatory cells from the periphery is noticeably absent. Though this disease bears a striking resemblance pathologically to the prion diseases (Brooks *et al.,* 1980; Hoffman *et al.,* 1982), CasBrE is a conventional virus that is readily inactivated by heat and detergents. There is no accumulation of protease-resistant "prion" protein (PrP) in the brain (Caughey and Portis, unpublished data), and PrP-knockout mice are still susceptible to CasBrE (Jolicoeur *et al.,* 1996). The primary lesion appears to represent swollen neuronal dendritic processes (Lynch *et al.,* 1991; Nagra *et al.,* 1992), which coalesce to form the holes visible with the light microscope, though neuronal dropout is responsible for some of the vacuoles. Curiously, early EM studies noted the surprising absence of visible virus particles associated with the degenerative lesions (Andrews and Gardner, 1974).

Over the past 20 years since the discovery of CasBrE, other murine retroviruses including additional members of the ecotropic host range group (McCarter *et al.,* 1977; Wong *et al.,* 1983; Kai and Furuta, 1984; Bilello *et al.,* 1986; Zachary *et al.,* 1986; Simonian *et al.,* 1990; Hoffman *et al.,* 1992; Czub *et al.,* 1995a) as well as recombinant polytropic (Buller *et al.,* 1990) and amphotropic (Munk *et al.,* 1997) viruses have been found to be neuropathogenic.

[1] To whom correspondence should be addressed at Rocky Mountain Labs, 903 South 4th St., Hamilton, MT 59840. Fax: (406) 363-9286. E-mail: jportis@nih.gov.

0042-6822/98 $25.00

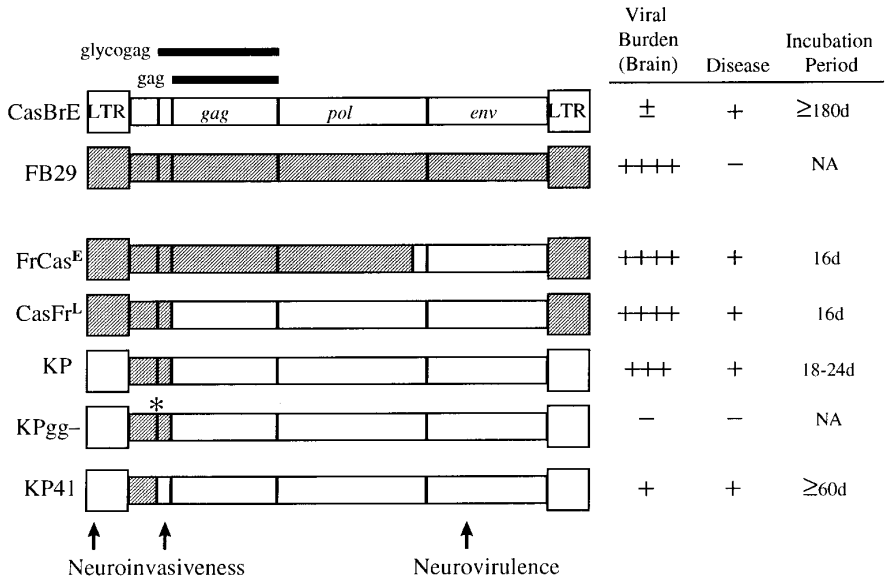

FIG. 1. Schematic summary of mapping studies illustrating the separation of sequences that influence neuroinvasiveness from those determining neurovirulence. All mice were inoculated intraperitoneally (ip) as neonates with the same dose of virus. The two parental viruses CasBrE and FB29 are shown at the top and various chimeric viruses below. The gag and glycosylated gag ("glycogag") proteins are shown above the genome of CasBrE. Glycogag is translated from an alternate initiation site upstream of, and in-frame with, the start codon of the gag proteins of the virion core. Thus, the coding sequences of gag and glycogag are identical, except for an extra 88 residues at the N terminus of glycogag, which represents the cytoplasmic tail of this integral membrane protein. The asterisk (*) above the genome of KPgg- indicates the location of mutations in the "Kozak" consensus sequence which interrupt translation of glycogag. "Viral burden" in the brain has been measured by several methods including Southern blot for viral DNA, immunoblot for viral protein, infectious center assays as well as immunohistochemistry. "Disease" is determined by observation of clinical status and "Incubation Period" represents the length of time (days) between inoculation and the first signs of clinical disease (NA-not applicable). This schematic illustrates several points: (1) Neurovirulence maps to the viral envelope gene. Unless a virus contains the CasBrE envelope gene, disease is not observed, irrespective of the magnitude of viral burden in the brain. (2) For viruses containing the CasBrE envelope gene, the tempo of the disease is directly related to viral burden in the brain. And (3) viral burden (neuroinvasiveness) is determined by sequences within the viral LTR and the cytoplasmic tail of glycogag.

The diseases caused by the ecotropic viruses appear essentially identical in clinical presentation and pathological features to that caused by CasBrE. The neurological disease caused by the polytropic viruses is distinct in that spongiform degeneration is minimal, glial activation is a dominant feature, and the disease is clinically manifested by initial hyper-excitability followed by ataxia and terminal immobility (Portis *et al.,* 1995). In addition, virus distribution in the brain is more restricted than that seen in the ecotropic viral diseases (Portis *et al.,* 1995). There are two additional retrovirus-induced diseases that are quite different from the others but will not be discussed here: an acute cerebrovascular hemorrhagic disease caused by a variant of Friend MuLV, TR13 (Park *et al.,* 1994) and a cognitive disorder associated with the immunodeficiency induced by the MAIDS virus complex LPBM5 (Kustova *et al.,* 1996).

Viral and host determinants of neuropathogenesis

Molecular cloning of CasBrE in the early 1980s (Jolicoeur *et al.,* 1983) made possible the construction of chimeric viral genomes, which demonstrated that the envelope gene harbors the principal determinants of neurovirulence (DesGroseillers *et al.,* 1984). Similar mapping studies on the other neurovirulent viruses have confirmed the role of *env* in neuropathogenesis (Yuen *et al.,* 1985; Portis *et al.,* 1990, 1995; Munk *et al.,* 1997). The envelope gene of oncornaviruses, like the lentiviruses, encodes two proteins, the surface glycoprotein (SU) and the transmembrane protein (TM) involved in receptor binding (Kabat, 1989; Heard and Danos, 1991) and fusion (Rein *et al.,* 1998) respectively. Mapping studies indicate that neurovirulence determinants of CasBrE are located in the SU component (Paquette *et al.,* 1989).

There are, however, other sequences that influence neuropathogenesis, and their location is illustrated in Fig. 1. While CasBrE is neuropathogenic, it is only weakly neuroinvasive and causes disease with a long incubation period. In contrast, a strain of Friend MuLV, FB29, is highly neuroinvasive but does not cause neurological disease. Introduction of the envelope gene of CasBrE into the genome of FB29 created the chimeric virus $FrCas^E$, which, like FB29, is highly neuroinvasive but also causes a rapidly fatal spongiform neurodegenerative disease (Portis *et al.,* 1990). For viruses carrying the CasBrE envelope gene, the tempo and severity of the disease is a function of viral burden in the brain (Czub *et al.,* 1992), and this in turn is determined by sequences from FB29.

Construction of additional chimeric genomes (Fig. 1, bottom) localized the relevant sequences from FB29 to the viral LTR, containing the promoter and transcriptional enhancers for viral RNA synthesis, and the 5′ leader sequence of the viral genome, 5′ of the initiation codon for the precursor of the *gag* proteins of the viral core (Portis *et al.,* 1994). This region, which has been called the 5′ untranslated region, actually encodes a protein called glycosylated *gag* (Fig. 1), a Type 2 integral membrane protein that is conserved in both murine (Tung *et al.,* 1976; Evans *et al.,* 1977) and feline (Neil *et al.,* 1980; Laprevotte *et al.,* 1984) oncornaviruses, but the function of which is unknown. Virus dissemination to the brain is affected by the sequencing encoding the N-terminal cytoplasmic tail of the protein (Portis *et al.,* 1994; Fujisawa *et al.,* 1998) (Fig. 1). These genetic studies, thus, have uncovered a dissociation between neuroinvasiveness and neurovirulence, both properties being important for the expression of the neuropathogenic phenotype.

How then do the viral LTR and glycosylated *gag* influence neuroinvasiveness? Both of these elements determine the kinetics of virus spread in peripheral nonneural tissues during the first few days after neonatal inoculation (Portis *et al.,* 1994). FB29 and $FrCas^E$ reach peak viremia titers within 5 day of inoculation, whereas CasBrE is significantly slower by 3 to 4 day (Czub *et al.,* 1992). This delay is critical because of a progressive resistance of the brain to infection, which appears postnatally as a function of age (Brooks *et al.,* 1981; Czub *et al.,* 1991). Resistance is first noted on postnatal Day 6 (P6) and is essentially complete by P10. It is independent of antiviral immunity and cannot be bypassed even by direct intracerebral inoculation of virus (Czub *et al.,* 1991). The mechanism of resistance is as yet unclear but may involve a loss of susceptibility of brain microvascular endothelial cells (Lynch *et al.,* 1995), the portal of entry of these viruses into the brain (Swarz *et al.,* 1981; Pitts *et al.,* 1987; Czub *et al.,* 1994). Although neuroinvasion from the periphery is restricted, local spread of virus within the brain parenchyma (i.e., beyond the microvasculature) continues, but at a relatively slow pace (Czub *et al.,* 1994; Lynch *et al.,* 1995; Robertson *et al.,* 1997). Thus, viral burden in the brain can be viewed at two levels, initial neuroinvasion from the periphery, dependent on susceptibility of brain endothelial cells, and the subsequent virus spread within the brain.

By virtue of the age-dependent resistance of the brain, neuroinvasion is influenced by any factors that affect the extent of virus spread in the periphery during the first few days after neonatal inoculation. This is accomplished artificially by altering the dose of the inoculum (Brooks *et al.,* 1979; Hoffman *et al.,* 1981; Czub *et al.,* 1992) or the time of inoculation (Hoffman *et al.,* 1981; Sharpe *et al.,* 1987; Czub *et al.,* 1991). Protective immunity appears not to play a significant role in limiting virus spread at this early time point. Mice inoculated with oncornaviruses as neonates exhibit markedly restricted immune responses with no detectible protective humoral or cellular immunity (Huebner *et al.,* 1971; Hoffman *et al.,* 1984; Korostoff *et al.,* 1990; Sarzotti *et al.,* 1996). The hyporesponsiveness is virus-specific and, for some viruses, may be related to infection of the thymus during the perinatal period (Gaulton, 1998). Genetic resistance mechanisms have been described, and these do function during the neonatal period. Resistance genes have been identified in wild mice [*Akvr*-1 (Gardner *et al.,* 1980) and FV-4 (Ikeda and Sugimura, 1989)] as well as laboratory mice (RMCF; Ruscetti *et al.,* 1981; Buller *et al.,* 1988). These genes encode endogenous retroviral envelope proteins that function by interfering with receptor availability. Interestingly, receptor blockade can be achieved artificially by prior inoculation of laboratory mice with nonpathogenic retroviruses that use the same receptor as the pathogenic strain (Corbin and Sitbon, 1993; Czub *et al.,* 1995b). FV-1, a recently cloned resistance gene (Best *et al.,* 1996) that produces strong resistance to neurological disease induced by CasBrE (Oldstone *et al.,* 1980), encodes an endogenous retroviral *gag* protein, which interferes with an early postentry step in the virus life cycle (Jolicoeur and Baltimore, 1976). Mouse genetic studies have revealed the existence of other resistance genes (Oldstone *et al.,* 1980; Hoffman and Morse, 1985; Buller *et al.,* 1990), though they have not been further characterized.

The aforementioned viral and host determinants either promote or restrict access of virus to the brain. Another type of host effect was observed during experiments in which the highly neurovirulent virus $FrCas^E$ (Fig. 1) was inoculated *in utero* into midgestation embryos (Lynch and Portis, 1993). Because of the rapidity of spread of the virus, it was anticipated that the fetuses might not survive to parturition. The virus, indeed, infected the brain *in utero* and achieved levels of viral burden in the brain far exceeding that seen in neonatally inoculated mice. Yet pathological and clinical signs of disease appeared at the same postnatal days as observed in neonatally inoculated mice (P10 and P15, respectively), indicating that P10 was a critical time point before which the brain appeared refractory to the neuropathogenic effects of virus infection. The nature of this developmental resistance is not known.

What cells are infected in the brain?

Because the mouse genome is replete with endogenous retroviral sequences (Best *et al.,* 1997), it was important in searching for the identity of the infected cells to use probes that would detect only the inoculated virus. Although there is extensive homology among murine oncornaviruses, the envelope gene as well as sequences of the U3 region of the viral LTR exhibit sufficient sequence diversity to generate reagents specific for CasBrE. CasBrE-specific nucleic acid probes (Rassart

et al., 1986; Portis *et al.,* 1990) and monoclonal antibodies (McAtee and Portis, 1985) have identified a variety of cells in the brain infected by CasBrE and its derivatives (Kay *et al.,* 1991; Lynch *et al.,* 1991). As mentioned above, the first cells to be infected are microvascular endothelial cells where the virus amplifies. The vascular infection, while impressive and sometimes productive of striking accumulations of virus particles (Oldstone *et al.,* 1977; Swarz *et al.,* 1981; Lynch *et al.,* 1991), appears not to disrupt the blood-brain barrier (Nagra *et al.,* 1992). Infected cells of the brain parenchyma are essentially of two types, microglial cells and neurons. Infection of neuroglia (astrocytes and oligodendrocytes) is seen (Oldstone *et al.,* 1977) but is rare (Gravel *et al.,* 1993). Infected neurons have been identified in the cerebellar cortex, hippocampus, and olfactory bulb (Kay *et al.,* 1991; Lynch *et al.,* 1991; Gravel *et al.,* 1993), populations of neurons that are distinguished by their proliferation postnatally (Miale and Sidman, 1961). Interestingly, although infection of these neuronal populations is fully permissive (Lynch *et al.,* 1991), these cells never exhibit signs of cytopathology (Lynch *et al.,* 1991; Gravel *et al.,* 1993).

There is some disagreement in the literature concerning the question of whether neurons within the areas of the CNS exhibiting neurodegenerative lesions are infected by this virus. This is not a trivial issue because these neurons are thought to be largely nonmitotic after midgestation and their infection by CasBrE (inoculated into neonates) would suggest that this virus was unique among murine oncornaviruses in productively infecting nondividing cells (Oldstone *et al.,* 1977, 1983). Indeed, initial ultrastructural studies on paralyzed CasBrE-infected mice (Gardner *et al.,* 1973; Andrews and Gardner, 1974) revealed C-type virus budding into intracellular vesicles of motor neurons in the ventral horns of the spinal cord, a finding that has been confirmed by others (Oldstone *et al.,* 1977, 1980, 1983; Swarz *et al.,* 1981) including ourselves (Portis, 1990). In addition, oncornaviral proteins have been found in these cells by immunohistochemical techniques using detecting reagents such as polyclonal antisera to CasBrE whole virus (Oldstone *et al.,* 1977) or viral proteins (Morey and Wiley, 1990; Sharpe *et al.,* 1990). However, neither EM nor immunodetection with polyclonal antisera would distinguish the inoculated virus from an endogenous retrovirus expressed perhaps as a consequence of neuronal injury. Polyclonal antisera to oncornaviral proteins are characteristically broadly reactive with a variety of both exogenous and endogenous viruses. In contrast to these findings, application of CasBrE-specific probes to address this question in both slow and rapid forms of the disease has consistently failed to detect expression of the inoculated virus in these neurons (Kay *et al.,* 1991; Lynch *et al.,* 1991; Gravel *et al.,* 1993). In the rapid disease caused by derivatives of CasBrE, evidence of viral protein expression in these neurons has also not been forthcoming, even using broadly reactive reagents (see Fig. 2). Thus, it appears more likely that virus expression detected in these nondividing neurons is not the result of infection but instead represents the activation of an endogenous provirus harbored in the germline of the mouse and may be a late occurrence seen only in more chronic forms of disease. CasBrE, thus, would appear to obey the same rules as the other oncornviruses in requiring cell division for productive infection (Hajihosseini *et al.,* 1993), a conclusion that is supported by *in vitro* studies (Lynch *et al.,* 1994). Collectively these observa-

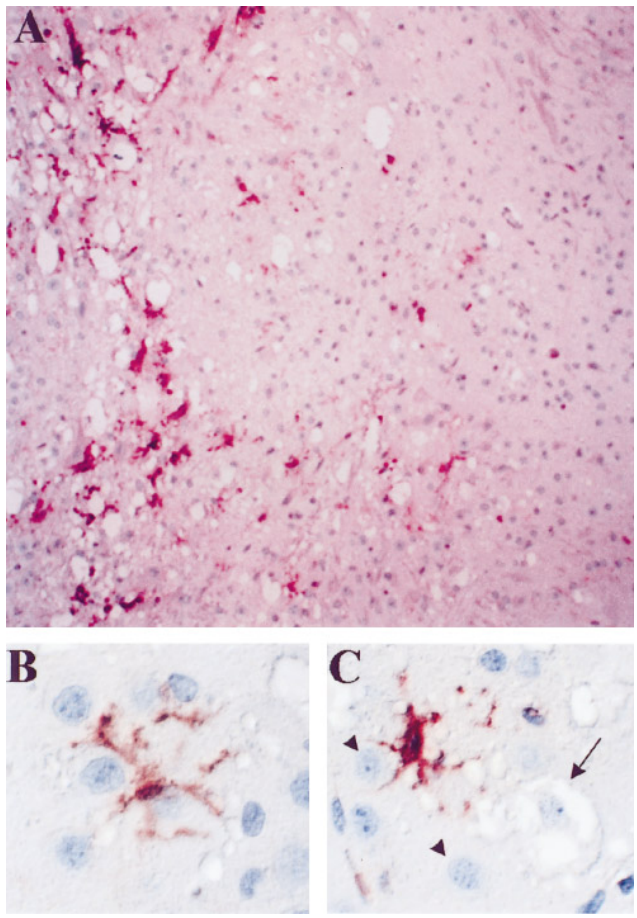

FIG. 2. These are photomicrographs of the brain stem of a mouse neonatally inoculated ip with the CasBrE derivative KP (Fig. 1) and killed in the terminal stage of clinical disease 22 days later. Paraffin embedded sections were subjected to antigen-retrieval (Shi *et al.,* 1993) and stained with a polyclonal anti-SU antiserum using immunoperoxidase. Spongiform degeneration, seen at low magnification as collections of holes in the tissue (A), colocalizes with the infected cells (stained red), which, in this location, consist primarily of microglia (Lynch *et al.,* 1991; Gravel *et al.,* 1993). At higher power, the processes of infected microglia are seen to be associated with microvacuoles in the neuropil between neurons (B) and with a neuron cell body exhibiting cytoplasmic swelling characteristic of "hydropic" degeneration (C, arrow). The degenerating cell body is identified as a neuron by its nuclear morphology, which resembles that of other intact neurons in the same field (C, arrowheads). Note that the neurons are negative for viral envelope protein. Magnifications: (A), ×30; and (B and C), ×125 before enlargement.

tions indicate that, although some populations of neurons which divide postnatally are productively infected by CasBrE and its derivatives, this infection is not cytotoxic. In contrast, the neurons that degenerate appear not to be infected, leading one to the conclusion that the neuronal injury induced by this virus is a consequence of indirect mechanisms.

Infected microglial cells are abundant in this disease (Lynch *et al.,* 1991; Gravel *et al.,* 1993), and it is these cells that co-localize with the spongiform lesions (Fig. 2). Unlike the neuroglia, which are of neuroectodermal origin, microglial cells are of mesodermal origin (Hickey *et al.,* 1992; Theele and Streit, 1993) and are thought to be the resident macrophages of the brain. The importance of microglial infection has been strengthened by similar findings in the diseases caused by a neurovirulent variant of Moloney MuLV, ts1 (Baszler and Zachary, 1990, 1991), a rat adapted variant of Friend MuLV, NT-40 (Czub *et al.,* 1995a) as well as the recombinant amphotropic virus, Mo-AmphoV (Munk *et al.,* 1997). Furthermore, studies of a neurovirulent polytropic virus Fr98 (Portis *et al.,* 1995) and its derivatives (Hasenkrug *et al.,* 1996) has revealed a direct relationship between the frequency of infected microglia and the occurrence of clinical disease (Robertson *et al.,* 1997).

Microglial cells and spongiform degeneration

To address directly the importance of microglia in neuropathogenesis, infected primary microglial cells were implanted into the brains of mice at P10, a time point at which spread of virus to recipient brain cells is restricted. The virus used for these studies was $FrCas^E$, the highly neuropathogenic derivative of CasBrE (Fig. 1). The implanted microglia assumed an highly ramified morphology, persisted for several weeks, migrated within the ipsi- and contralateral hemispheres and continued to express viral proteins. Three weeks after implant, focal spongiform lesions were detected in the immediate vicinity of the infected microglia (Lynch *et al.,* 1995). Implantation of uninfected microglia failed to induce lesions. These experiments indicated that infected microglia alone were sufficient for induction of lesions, but did not reveal whether they were necessary or obligate participants.

To address this issue, a neural progenitor cell line (C17–2) (Ryder *et al.,* 1990) was used which, when transplanted into the neonatal mouse brain intraventricularly, is incorporated into the subventricular germinal matrix and is disseminated widely throughout the brain (Snyder *et al.,* 1992). These cells differentiate into neurons, astrocytes, and oligodendrocytes, which become integrated into the normal CNS cytoarchitecture and persist for extended periods (Snyder *et al.,* 1997; Taylor and Snyder, 1997). What is significant, however, is that C17–2 are of neuroectodermal origin and do not differentiate into microglia, providing a means of expressing virus in the brain from a source which is unrelated to microglial cells. The C17–2 cells were productively infected with a highly neurovirulent derivative of CasBrE [$CasFr^L$ (Fig. 1)] and injected intraventricularly into neonatal FV-1 incompatible mice. As mentioned above, FV-1 is a resistance gene that interferes with an early postentry step in the replication cycle. Although these virus-producing cells engrafted in regions of the brain that were susceptible to the virus, in numbers comparable to that seen in neonatally inoculated mice, lesions were not observed (Lynch *et al.,* 1996). In contrast, $FrCas^E$, which was FV-1 compatible with the recipient mouse and spread unrestricted to recipient microglia, induced typical spongiform lesions. In a separate experiment, C17–2 cells expressing only the envelope protein of CasBrE were implanted. Here no infectious virus was present, only cells expressing high and sustained levels of envelope protein. No lesions were observed in these brains (Lynch *et al.,* 1996). We can conclude from these implantation experiments that (1) infected microglial cells are both necessary and sufficient for induction of the spongiform lesions; (2) the envelope protein of CasBrE is not directly neurotoxic *in vivo;* and (3) neurotoxicity is not a consequence of virus/receptor interactions at the cell surface, a conclusion drawn from the fact that FV-1 restriction occurs at a postentry step. In short, the results of the implants would argue that it is productive infection of microglial cells that is responsible for the neurovirulence of these viruses.

Role of the viral envelope protein

Clearly, the envelope gene harbors important determinants of neurovirulence (Fig. 1). If the envelope protein is not neurotoxic, then what is its role in neuropathogenesis? Some clues have come from mapping studies. In two unrelated viruses, PVC-211 (ecotropic) (Masuda *et al.,* 1993) and Fr98 (polytropic) (Hasenkrug *et al.,* 1996), the relevant sequences within the envelope gene that determine neurovirulence are located in the N-terminal half of the surface glycoprotein (SU). This part of the protein contains the receptor-binding domain (Battini *et al.,* 1995; MacKrell *et al.,* 1996; Davey *et al.,* 1997). Based on the recently crystallized receptor binding domain of the Friend MuLV SU protein (Fass *et al.,* 1997), one of two critical residues of PVC-211 (G_{116}) appears to be close to the putative receptor binding surface of the molecule. Interestingly, these residues enhance the ability of PVC-211 to infect brain capillary endothelial cells (Masuda *et al.,* 1996) and thus are likely to have a strong influence on the extent of neuroinvasion occurring during the perinatal period. In the case of Fr98, the sequence of the N-terminal half of SU influences the kinetics of virus spread within the brain parenchyma, specifically to microglial cells during the period 3 to 8 week postinocula-

tion (Hasenkrug *et al.,* 1996; Robertson *et al.,* 1997). Thus, in both cases, sequences that influenced neurovirulence also appear to influence viral tropism in the brain, and it is possible that this effect alone could explain the role of the viral envelope protein in this disease.

While the implant experiments indicated that the envelope protein was not neurotoxic when expressed in the brain by C17–2 cells, there is some evidence that expression of only the viral envelope protein in the brain may be neuropathogenic under certain conditions. Mice carrying either the CasBrE (Kay *et al.,* 1993) or ts-1 Moloney (Yu *et al.,* 1997) envelope genes as transgenes, driven by the respective viral promoters, do indeed develop focal spongiform lesions. In both cases, expression levels in the brain were extremely low (detectable only by RNAase protection assay), incubation periods were very long (1 to 2 year) and clinical manifestations were minimal at best. The difference in the results obtained with these two approaches (implants *vs* transgenic mice) might be explained by the identity of the cells expressing the protein. In the C17–2 implants, the cells were of neuroectodermal origin. In the transgenics, though the *env*-expressing cells were not actually identified, the viral promoters used in both cases function well in microglial cells (Baszler and Zachary, 1991; Gravel *et al.,* 1993). Thus, together these experiments suggest that expression of the envelope gene alone in microglial cells might be sufficient to induce lesions. Whether the sequence of the envelope protein expressed in microglia influences neurovirulence was not addressed in these experiments. It is also unclear that the effect is *env*-specific at all. Thus, expression of other viral genes in microglia may be equally neuropathogenic. These questions can perhaps best be addressed by studying viruses such as FB29 (Fig. 1) which are neuroinvasive, but not neurovirulent.

Does glial activation play a role in disease induction?

Through manipulation of the virus and the host it has been possible to separate factors relevant to neurovirulence of the murine oncornaviruses from those that represent epiphenomena. One pathological change that is observed in all the more chronic forms of neurological disease caused by these viruses is the occurrence of reactive gliosis, manifested by immunohistochemical signs of microglial and astocytic activation. Microglia are exquisitely sensitive to even subtle perturbations of the nervous system, transforming from highly ramified "quiescent" cells to actively phagocytic "amoeboid" microglia, which can present antigen and participate in local immune responses (Kreutzberg, 1996; Streit, 1996). Microglial activation appears to be the first line of defense of the brain to invading microbes. Thus, it is not surprising that these cells would be activated in response to neuroinvasive viruses. However, it has been proposed that glial activation, and specifically microglial activation, is implicated causally in a number of chronic degenerative diseases such as HIV encephalopathy (Lipton, 1997; Gendelman and Tardieu, 1994), Alzheimer's disease (Martin *et al.,* 1994; Frautschy *et al.,* 1998), and prion diseases (Brown *et al.,* 1996). Indeed, the best correlate of the dementia associated with HIV infection of the brain is not the frequency of virus infected cells, but the frequency of activated macrophage/microglial cells (Glass *et al.,* 1995; Tyor *et al.,* 1995). These cells can secrete a variety neurotoxic substances *in vitro* including a cytokines, reactive oxygen species and NMDA receptor agonists (Lipton, 1997). However, some of the cytokines expressed by these cells also have neuroprotective effects (Barger *et al.,* 1995; Strijbos and Rothwell, 1995; Bruce *et al.,* 1996) and activated microglia produce bona fide neurtrophins (Elkabes *et al.,* 1996). Thus, it is not always clear whether the activation of these cells *in vivo* is harmful or helpful.

In the case of the murine oncornaviruses, there is evidence from several quarters that neurovirulence may be separable from microglial activation. (1) Both neurovirulent and avirulent polytropic viruses induce intense microglial activation in the same regions of the brain (Robertson *et al.,* 1997), indicating that glial activation alone is not sufficient to precipitate clinical disease. (2) In the microglial implantation experiments described above, focal spongiform lesions were spatially associated with infected microglial cells, but these cells did not express cell-surface activation markers (Lynch *et al.,* 1995). And (3) $FrCas^E$, the highly neurovirulent derivative of CasBrE, infects large numbers of microglial cells and induces a rapidly fatal spongiform degeneration that is widespread already at 14 days postinoculation, yet, again, without expression of cell-surface markers indicative of microglial activation (Lynch *et al.,* 1991; Czub *et al.,* 1994). Simply diluting the virus inoculum so as to delay the onset of clinical disease, resulted in the appearance of intense gliosis (Czub *et al.,* 1994), indicating that $FrCas^E$ is capable of inducing glial activation so long as the mice lived long enough.

Concluding remarks

Although the function of microglia in initiating local immune responses and as the scavengers of the brain is well accepted, these cells also appear to play important, but poorly understood, roles in neural support. The implantation studies outlined above indicate that the neurovirulence of the murine oncornaviruses is tightly linked to the expression of one or more viral genes by microglial cells but that the products of those genes are not themselves neurotoxic. Certainly this scenario is consistent with the hypothesis that infection might induce the expression of neurotoxins by these cells. However, the unexpected lack of correlation with microglial activation

leaves open the possibility that the neurovirulence of these viruses is effected by a loss of microglial function. Gaining a better understanding of the effect of these viruses on the biology of the microglial cell *in vivo* is likely to teach us as much about the pathogenesis of these brain diseases as it is about the special functions of these cells.

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