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# Neurovirology

## Viruses and the Brain

Edited by
Michael J. Buchmeier
Iain L. Campbell

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## VOLUME 56

## NEUROVIROLOGY

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# PREFACE

The idea for this volume arose during discussions between the editors in the spring of 1999 and was fueled by discussions with our colleagues here at The Scripps Research Institute and elsewhere. It was clear when this project began that long held beliefs and assumptions about the central nervous system, its accessibility to immune surveillance, and its response to infection required reassessment in light of advances in neurobiology and neuroimmunology. One goal of this book is to highlight the contemporary thinking in these areas. As is the case with any such endeavor we run the risk of omitting important viewpoints, or interpretations, but we are confident that the contributing authors have taken great care to present well-reasoned and balanced arguments on some of the most important issues existing in neurovirology.

Preparing a book such as this requires considerable help. The editors would like to express our appreciation first to all of the contributing authors and to our colleague Dr. Floyd Bloom. We would also like to acknowledge the efforts of Aaron Johnson and Traci John of Academic Press. Finally we would like to thank our spouses, Julia and Nancy, who have shown patience with our temporary insanity as this volume has evolved.

Iain L. Campbell
Michael J. Buchmeier

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# INTRODUCTION

The National Institutes of Health designated the 1990s as the decade of the brain. The intent of this notable action was to stimulate research in the neurosciences, thus it came to be that we witnessed a quantum increase in our knowledge of how the brain and the central nervous system (CNS) respond to viral infection, how viral infections trigger and sustain frank and subtle neuropathologies, and how these interactions manifest as degenerative and cognitive disorders. Viruses are among the most common environmental influences we encounter in the normal human life span, and these insidious agents are well adapted to infect and persist within the CNS. As a result of eons of evolution with viruses, the mammalian brain has in turn evolved unique mechanisms of defense to combat and control infection. Principal amongst these strategies are: First, a blood-brain barrier in which the endothelial cells form tight junctions and which serves as an efficient barrier to infectious agents and to cellular and macromolecular components of the immune system. Second, a relative deficiency of proteins needed for the recognition of antigen such as major histocompatibility complex molecules and activation of immune cells. Third, the absence of "professional" antigen presenting cells such as dendritic cells, and finally, the lack of a lymphatic drainage. However, in spite of these properties, it can no longer be presumed that the brain is an immunologically "privileged" site. Under the appropriate circumstances activated immune cells can and do enter and traffic through the brain. These unusual properties of the brain have many implications for the host and for any virus that may eventually gain entry. Viruses such as rabies and poliovirus that enter the brain by axonal transport and are sequestered inside immunologically incompetent neuronal cells, free to replicate and spread in an unfettered manner, eventually lysing their host cell and causing severe morbidity and mortality. At the other extreme, the unleashed fury of a targeted antiviral immune response in the brain, as occurs in, for example, HSV encephalitis, may also bring about the swift demise of the host. Between these extremes are viruses such as human immunodeficiency virus-1 that can enter the

brain and persist for life, causing limited damage and provoking only mild to moderate host responses. In all, the recognition and elaboration of these mechanisms of defense and their consequences forms the crux of much of contemporary viral pathogenesis, and is the foundation for this volume.

At the beginning of this new millennium we find that the spectacular advances of the previous decade in the fields of genetics, immunology, and molecular biology and their application to virology have catapulted our understanding of the etiology and the pathogenesis of many viral diseases of the CNS. This volume brings together a distinguished group of authors to explore the many facets of contemporary neurovirology. As they make clear in this book, viral–host interactions in the brain are complex and dynamic and often on shifting ground. The environment of the CNS may not only mould the nature and function of antiviral T cells and other leucocytes that enter the brain, but it may also apply selective pressure on the infectious agent to rapidly evolve to escape the unwanted attentions of the host immune surveillance. A number of authors focus on more well established models of neurological infections to highlight the diverse mechanisms and consequences of persistent virus infection of the brain. Others provide fascinating insights into the discovery of new viral links to established human psychiatric and demyelinating diseases. The enigmatic spongiform encephalopathies, scrapie and BSE, are now thought to be due not to a classical viral agent but rather to an aberrant or "rogue" protein termed a prion. As outlined in this volume, elegant studies both *in vitro* and *in vivo* are removing the veil of mystery that has shrouded our understanding of the pathogenesis of these neurological diseases. Finally, we are beginning to see some benefits flowing out of the many advances in molecular virology with two examples highlighted in this volume illustrating the power of this technology. The unique specificities of viruses are already being harnessed to map circuitry within the brain and to deliver genetic material to specific populations of cells within the brain. With the adaptation of modern genomic technologies the future is certain to hold both new problems and answers to old questions about the nature of the interaction between viruses and the brain.

Iain L. Campbell
Michael J. Buchmeier
*La Jolla, California, 2001*

# BASIC MODELS

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# GENETIC DETERMINANTS OF NEUROVIRULENCE OF MURINE ONCORNAVIRUSES

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## I. Introduction

Much of our knowledge of mammalian retroviruses had its origins in the derivation of inbred strains of mice (Slye, 1914). It was through the use of the C3H strain, inbred for its high incidence of mammary adenocarcinoma (Strong, 1935), but its low incidence of spontaneous leukemia, that Gross identified the first murine leukemia virus (MuLV)(Gross, 1951). However, the induction of cancers by these viruses in highly inbred mice was considered to be somewhat of a laboratory artifact of inbreeding, and there was great interest in determining whether these viruses might also be the cause of cancers in outbred animals and perhaps humans. In the early 1970s, Murray Gardner and associates began a search for leukemia viruses in outbred animals (Gardner, 1994); and over a 10-year period they collected, and observed in the laboratory, over 10,000 mice from 15 different areas of southern

California (Gardner *et al.,* 1976; Gardner, 1978). Although mice from the majority of the collection areas were virus negative, they discovered several colonies that had an unusually high incidence of nonthymic lymphoma (Bryant *et al.,* 1981) and expressed lifelong high levels of infectious MuLV. Some of these mice also developed a neurologic disease manifested by hind limb paralysis (Gardner *et al.,* 1973). The field isolates were found to contain two types of murine retroviruses belonging to the ecotropic and amphotropic host range groups (Hartley and Rowe, 1976; Rasheed *et al.,* 1976). Ecotropic viruses infected only rodent cells *in vitro,* whereas the amphotropic viruses infected cells of a variety of species, including mice. The host range of MuLV is determined by the sequence of the envelope gene (Battini *et al.,* 1992). Based on viral interference analyses, these two viruses were shown to use different receptors to enter cells (Hartley and Rowe, 1976; Rein, 1982), and these receptors have now been cloned and characterized (Albritton *et al.,* 1989; Miller *et al.,* 1994). The ecotropic viruses were found to induce both the nonthymic lymphomas and the paralytic syndrome (Rasheed *et al.,* 1976; Rasheed *et al.,* 1983). The prototypic virus in this group was isolated from the brain of a mouse captured at a squab farm near Lake Casitas in southern California and was named CasBrM (Hartley *et al.,* 1976), the "M" designating it as mouse-tropic. When the nomenclature of mouse-tropic viruses was changed to the ecotropic designation, the name was changed to CasBrE.

Although the leukemia viruses first identified by Gross were integrated in the germ line as proviruses, this is not the case for the wild mouse viruses (Barbacid *et al.,* 1979; O'Neill *et al.,* 1987). Gardner and his colleagues found that these viruses are transmitted primarily from mother to offspring in the milk (Gardner *et al.,* 1979), although evidence of horizontal transmission among adults in external secretions is also seen (Gardner *et al.,* 1979; Portis *et al.,* 1987). Neonates become persistently infected and remain viremic for life. These mice are immunologically unresponsive to the virus. Thus, neither antiviral antibodies nor cytotoxic T lymphocytes are detectable at any time after neonatal inoculation, though more sensitive techniques have revealed evidence of weak antigen-specific $T_H2$ responses (Sarzotti *et al.,* 1996). Only in mice inoculated neonatally is the neurologic disease observed and in the wild the incidence is low (<20%) and the incubation period long (several months to more than a year) (Gardner *et al.,* 1973). Neurologic disease is virtually never seen in mice inoculated after they are 6–8 days of age (Hoffman *et al.,* 1981; Officer *et al.,* 1973). Although maturation of the immune response appears to be one component of this age-dependence (Hoffman *et al.,* 1981), this resistance has also

been observed in athymic nude mice (Czub *et al.,* 1991)—an issue which will be discussed further in Section V.

The occurrence of the ecotropic virus in only some of the colonies of wild mice appears to be the consequence of the segregation of a host resistance gene, FV-4 (Gardner *et al.,* 1980; Odaka *et al.,* 1981). Interestingly, FV-4 is a truncated replication-defective ecotropic provirus (Kozak *et al.,* 1984) that encodes an endogenous ecotropic envelope protein (Ikeda and Odaka, 1983; Limjoco *et al.,* 1993) and that restricts virus spread by blocking the ecotropic receptor (viral interference). This resistance gene is undoubtedly the remnant of a remote infection, the critical component of which was conserved in the mouse genome because it endowed the host with a selective advantage.

The vast majority of diseases caused by the oncornaviruses are proliferative in nature. Infected cells are induced to proliferate by a number of mechanisms. Friend virus (SFFV) encodes a truncated envelope protein that binds to the erythropoietin receptor (Li *et al.,* 1990), inducing polyclonal proliferation of erythroid progenitors. Envelope proteins of other MuLVs of the polytropic host range are thought to cause polyclonal T cell proliferation by binding to cell surface receptors linked to signal transduction pathways (Li and Baltimore, 1991). Diseases characterized by clonal proliferation, such as leukemias and lymphomas, are a consequence of the capacity of these viruses to integrate a DNA copy of their genome into the host cell genome, thereby activating or inactivating cellular genes involved in growth control (Rosenberg *et al.,* 1997). The lymphomas and leukemias caused by CasBrE are of this type (Bergeron *et al.,* 1991; Bergeron *et al.,* 1993). Although much remains to be learned of the molecular details, at least conceptually, the role of virus in these proliferative diseases is understood. Even at the conceptual level, however, little is yet known of the mechanisms responsible for the neurologic diseases induced by these viruses.

Before we discuss these neurologic diseases, we should define a few terms, originally formulated by R. T. Johnson (1998), which will be used repeatedly in this chapter. "Neurovirulence" simply refers to the capacity of a virus to cause neurologic disease and does not imply any specific mechanisms. "Neuroinvasiveness" defines a virus's capacity to invade the nervous system, but does not imply any specific cellular tropism. "Neurotropism" means that a virus actually infects neurons. Thus, neurovirulence implies that the virus is neuroinvasive but does not always indicate that the virus is neurotropic. As will be discussed later (Section III), neuroinvasiveness does not always lead to neurovirulence.

## II. Clinicopathological Manifestations of Neurovirulent Murine Oncornavirus Infection

### *A. Ecotropic Viruses*

The initial clinical sign of the disease caused by CasBrE is an abnormal abduction reflex of the hind limbs that occurs when the mice are lifted by the tail; it is generally associated with an action tremor (Andrews and Gardner, 1974). As the disease progresses, the mice exhibit hind limb weakness and, finally, frank spastic paralysis that may also involve the forelimbs (Table I). At this stage there is also wasting associated with neurogenic atrophy of skeletal muscles (Gardner *et al.,* 1973). The predominant neuropathologic features are spongiosis (vacuolation) and gliosis involving preferentially the caudal regions of the nervous system. Demyelination has been observed (Andrews and Gardner, 1974; Oldstone *et al.,* 1977) but appears to be secondary to the damage to the neuronal perikarya. Whether neurons actually die and drop out in this disease has been debated. It is clear, however, from ultrastructural studies that neurons and glial cells undergo degenerative changes (Brooks *et al.,* 1980; Swarz *et al.,* 1981), and in mice with long-standing chronic disease, there is a depopulation of neurons, at least in the anterior horns of the spinal cord (Andrews and Gardner, 1974; Oldstone *et al.,* 1980).

Ultrastructurally, the vacuolar changed induced by CasBrE (Andrews *et al.,* 1974; Swarz *et al.,* 1981) bears a striking resemblance to scrapie and the other TSEs (Hoffman *et al.,* 1982; Lampert *et al.,* 1972). Vacuoles appear to represent swollen postsynaptic terminals (dendrites) and are often filled with membranous material. Interestingly, it was observed early on that virus particles appeared not to be associated with these vacuoles (Andrews and Gardner, 1974; Swarz *et al.,* 1981). Instead, in a number of ultrastructural studies, virus budding was observed primarily in the central nervous system (CNS) microvasculature (Hoffman *et al.,* 1992; Oldstone *et al.,* 1977; Pitts *et al.,* 1987), in both endothelial cells and pericytes (probably perivascular macrophages). In addition, however, budding virus was also noted in occasional large motor neurons of the ventral horns of the spinal cord (Andrews and Gardner, 1974; Gardner *et al.,* 1973; Oldstone *et al.,* 1977; Oldstone *et al.,* 1980; Oldstone *et al.,* 1983). Virus in these cells appeared to be budding primarily into intracellular vesicles, possibly endoplasmic reticulum. It was noted however, in the initial studies on the pathogenesis of CasBrE that the neurons exhibiting intracellular virus budding appeared not to be undergoing degeneration, and indeed, the neurons which did exhibit degenerative changes did not harbor such

virus particles (Andrews and Gardner, 1974). Furthermore, the intraneuronal virions were observed only in mice with chronic forms of disease and were never seen in mice developing the disease after short incubation periods (Swarz *et al.,* 1981). These virus particles are likely the result of the activation of endogenous retroviruses and thus probably represent an epiphenomenon (see section X). These discrepancies led Andrews and Gardner (1974), in their original ultrastructural study of this disease, to suggest that the neuropathogenesis of CasBrE was a consequence of indirect effects of virus infection, a prediction which now seems to be correct. The nature of these effects is still a matter of speculation, but the capacity to manipulate both the viral genome and the host has brought us closer to understanding, at the molecular level, the virus–host interactions that lead to neuropathology.

Since the discovery of the wild-mouse ecotropic viruses, a number of other neurovirulent ecotropic viruses have been identified, including Moloney MuLV ts-1 (Wong *et al.,* 1983); Friend MuLV PVC211 (Kai and Furuta, 1984; Masuda *et al.,* 1992); and NT40 (Czub *et al.,* 1995). All cause spongiform encephalomyelopathies indistinguishable clinically and pathologically from the disease caused by CasBrE. While ts-1 and CasBrE are pathogenic only for mice, PVC211 and NT40 cause disease in rats, having been selected by serial passage in that species.

### *B. Polytropic Viruses*

Polytropic viruses have a broader host range than the ecotropic viruses but are distinct from the wild-mouse amphotropic viruses, which also have a wide host range (Cloyd *et al.,* 1985). The polytropic receptor has recently been cloned by three independent groups (Battini *et al.,* 1999; Tailor *et al.,* 1999; Yang *et al.,* 1999) and is also structurally and functionally distinct from the ecotropic and amphotropic receptors. These viruses are recombinants between ecotropic viruses and endogenous retroviral sequences resident in the mouse genome (Stoye and Coffin, 1987). The neurovirulent polytropic virus FMCF98 causes a disease which is different both clinically and pathologically from that caused by the ecotropic viruses (Table I). Mice manifest early hyperexcitability that characterizes them as “popcorn” mice (Portis *et al.,* 1995). The mice jump out of the cage at the slightest auditory stimuli, such as snapping of the finger. This early stage is followed, within days or weeks, by evidence of imbalance, ataxia, and, finally, immobility associated with generalized seizures. Pathologically, there is an intense gliosis involving both astrocytes and microglia, but spongiosis is a rare and inconsistent finding (Portis *et al.,* 1995).

### *C. Other Neurovirulent Viruses*

All of these diseases are manifested by clinical signs that are easily recognized (e.g., paralysis, tremor, seizures). There is another murine retroviral disease that is manifested primarily by cognitive deficits. The virus is LP-BM5, a virus complex composed of a replication-defective virus containing extensive deletions (Aziz *et al.,* 1989) and a replication-competent helper virus. Mice inoculated with this virus complex as adults develop a lymphoproliferative disease associated with profound immunosuppression (Mosier *et al.,* 1985), and with opportunistic infections (Gazzinelli *et al.,* 1992). These effects are due to the presence of the defective virus, not the helper (Aziz *et al.,* 1989). The cognitive deficit is manifested by abnormalities in spatial learning (Sei *et al.,* 1992) and is associated with evidence of microglial and astrocytic activation (Kustova *et al.,* 1996); abnormalities in neurotransmitter levels (Espey *et al.,* 1998; Kustova *et al.,* 1997); signs of neuronal degeneration; and synaptic remodeling (Kustova *et al.,* 1998) (Table I).

In addition to the neurodegenerative diseases, murine oncornaviruses also cause acute fatal cerebrovascular hemorrhage. Intracerebral hemorrhage was found to be the cause of death of some mice infected with CasBrE (Portis *et al.,* 1990), and more recently a mutant of Friend MuLV (TR1.3) was found to induce a rapid widely disseminated cerebrovascular hemorrhage (Park *et al.,* 1993) (Table I). The hemorrhages caused by TR1.3 are the consequence of the fusigenicity of the envelope protein for endothelial cells in the brain, resulting in a disruption of vascular integrity (Park *et al.,* 1994b, 1994a).

## III. Mapping of Viral Determinants of Neurologic Disease

Murine oncornaviruses are simple retroviruses with three structural genes (*gag, pol,* and *env*) that are bounded by long terminal repeats (LTRs) at the ends of the genome that carry the transcriptional promoter and termination sequences (Miller, 1997) (Fig. 1). The *gag* gene encodes the core proteins of the virus. The *pol* gene encodes the viral protease, polymerase, and integrase involved in replication of the viral genome. The *Env* proteins comprise the surface of the virion and are involved in receptor binding and fusion, both functions of which are critical for virus entry into the host cell. Between the *gag* gene and the 5′ LTR is the "5′ leader" sequence that contains the primer binding site, splice donor, and packaging sequences of the viral RNA (Fig. 1).

Table II is a compilation of the results of genetic studies in which viral determinants of neurovirulence have been mapped. One common

TABLE I
MANIFESTATIONS OF NEUROVIRULENCE

| Virus | Host range | Clinical | Pathology | Reference |
|---|---|---|---|---|
| CasBrE | Ecotropic | Paralysis Tremor | Spongiosis Gliosis | Andrews and Gardner (1974) |
| CasBrE | Ecotropic | Early death | Cerebral hemorrhage | Portis *et al.* (1990) |
| Friend MuLV; PVC211 | Ecotropic | Paralysis, tremor | Spongiosis, Gliosis | Hoffman *et al.* (1992) |
| Moloney MuLV; ts-1 | Ecotropic | Paralysis, tremor | Spongiosis, Gliosis | Zachary *et al.* (1986) |
| Friend MuLV; NT-40 | Ecotropic | Paralysis, tremor | Spongiosis, Gliosis | Czub *et al.* (1995) |
| Friend MuLV; TR1.3 | Ecotropic | Early death | Cerebral hemorrhage | Park *et al.* (1993) |
| LPBM5[a] | Ecotropic | Cognitive deficit | Gliosis | Kustova *et al.* (1996); Sei *et al.* (1992) |
| MoAmpho V[b] | Amphotropic | Paralysis, tremor | Spongiosis, gliosis | Munk *et al.* (1997) |
| FMCF98 | Polytropic | Hyper-excitability, ataxia | Gliosis | Portis *et al.* (1995) |

[a] Virus complex consisting of replication-competent ecotropic helper virus and replication-defective virus (Aziz *et al.,* 1989).

[b] Chimeric virus consisting of Moloney MuLV with envelope sequences derived from amphotropic virus 4070A.

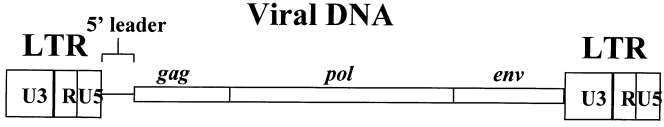


FIG 1. Schematic diagram of a typical murine oncornavirus genome. Represented is the organization of the viral DNA as it would exist as an integrated provirus in the genome of an infected cell. The long terminal repeat (LTR) at the 5′ end contains the transcriptional promoter (U3) and the LTR at the 3′ end contains the polyadenylation signal. The 5′ leader sequence contains the primer binding site for reverse transcription, and the packaging sequence required for proper incorporation of viral RNA into virus particles. The three structural genes are shown as blocks.

TABLE II
VIRAL DETERMINANTS OF NEUROVIRULENCE

| Virus | Sequences | Reference |
|---|---|---|
| CasBrE | *env* | DesGroseillers *et al.* (1984); Paquette *et al.* (1989) |
| CasBrE | LTR | DesGroseillers *et al.* (1985); Portis *et al.* (1991) |
| CasBrE | 5′ leader | Portis *et al.* (1994) |
| MoMuLV ts-1 | *env* | Szurek *et al.* (1988) |
| FMuLV PVC211 | LTR, *gag, env* | Masuda *et al.* (1993) |
| FMuLV TR1.3 | *env* | Park *et al.* (1994b) |
| MoAmphoV | *env,* non-*env*[a] | Munk *et al.* (1997) |
| FMCF98 | *env* | Portis *et al.* (1995) |

[a] The neuropathogenicity of MoAmpho V is a consequence of the combination of the envelope gene of amphotropic virus 4070A and the rest of the genome from Moloney MuLV. Neither of the parental viruses is neurovirulent (see the text).

feature for all of the viruses listed is the importance of the viral envelope gene in neurovirulence. It is also clear, however, that the envelope gene is not alone responsible. The sequence of the viral LTR, the 5′-leader sequence of the viral genome, as well as the *gag* gene, have also been shown to have an effect. This point is exemplified by the following observation: The virus MoAmpho V, listed in Tables I and II, is a chimeric virus consisting of the envelope gene from the wild-mouse amphotropic virus 4070A (Chattopadhyay *et al.,* 1981), and the rest of the genome from the Moloney MuLV clone MLV-K (Miller *et al.,* 1984). Neither of these parental viruses is neuropathogenic (DesGroseillers *et al.,* 1984; Munk *et al.,* 1997). Yet the chimeric virus caused a paralytic disease manifested by spongiosis and gliosis indistinguishable from that caused by CasBrE (Munk *et al.,* 1997; Munk *et al.,* 1998). This implies that the envelope gene of the parental amphotropic virus 4070A harbors sequences that can determine neurovirulence, given the appropriate context of other viral sequences—in this case donated by the Moloney MuLV parent.

In order to begin to simplify some of the complexities of the neurovirulent phenotype, we carried out a series of *in vivo* studies on CasBrE and derivatives thereof, using an inbred mouse strain (IRW) that had been shown to be highly susceptible to leukemogenesis induced by a variety of murine oncornaviruses. Like the disease seen in wild-mouse populations, our molecular clone of CasBrE induced neurologic disease in <10% of neonatally inoculated IRW mice, and in those mice that did develop the disease, incubation periods ranged from 3 to 6 months (Portis *et al.,*