Journal of Neuropathology and Experimental Neurology

Vol. 58, No. 12
December, 1999
pp. 1197–1206

Nidovirus Infections: Experimental Model Systems of Human Neurologic Diseases

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Abstract. The presence of terminally differentiated slow- and non-dividing cells in the central nervous system (CNS) provides a safe harbor for viral persistence and latency and constitutes a unique immunologic environment for viral infections. Studies of experimental model systems of viral infections of the CNS provide insight into mechanisms of viral persistence and immune-mediated pathology. Nidoviruses are comprised of 2 families of viruses, coronaviruses and arteriviruses, and are common pathogens of humans and a variety of animal species. Both families of viruses contain neurotropic strains that produce experimental neurologic diseases in rodents. These include acute meningitis and encephalitis; acute poliomyelitis; and chronic inflammatory, immune-mediated, demyelination. Coronavirus-induced demyelinating disease mimics many of the pathologic features of Multiple Sclerosis (MS).

Key Words: Arterivirus; Coronavirus; Demyelination; Encephalitis; Meningitis; Nidovirales; Torovirus.

INTRODUCTION

The CNS is considered "immunologically privileged" because the blood-brain barrier (BBB) is the most selective blood-organ barrier (1). Moreover, unlike other organs, the CNS is devoid of a lymphatic system and lacks resident lymphoid cells. The CNS has substantially reduced expression of major histocompatibility (MHC) class I antigens on its parenchymal cells, shielding them from recognition by cytotoxic T cells. The CNS also contains specialized resident immune cells, such as microglia and astrocytes that combine CNS-specific functions with functions that are considered traditional immune functions such as phagocytosis, antigen presentation, and cytokine secretion. The interaction between pathogens, especially viruses, and the CNS in this unique environment produces a number of pathologic conditions, which may be chronic and fatal. These conditions include subacute sclerosing panencephalitis (SSPE), progressive multifocal leukoencephalopathy (PML), HIV encephalitis, tropical spastic paraparesis/HTLV-1 associated myelopathy (TSP/HAM), and many others. The pathogenesis of these diseases is related to latent and persistent viral infections in nondividing, terminally differentiated cells.

Some immune-mediated CNS diseases, such as MS, have been indirectly linked to a viral etiology, but the exact correlation with viruses remains unknown (2). Multiple sclerosis is an autoimmune CNS disease, which may be mediated by autoreactive CD4+ T cells directed against myelin or oligodendroglial molecules. Epidemiological studies suggest that viral infections trigger clinical exacerbation in MS. Studying the relationship between viruses and MS can direct the development of new treatments for MS. Several animal models of virus-induced demyelination have been very helpful in elucidating induction mechanisms of demyelination following infections with ubiquitous viruses (3). Among the most studied animal models of virus-induced demyelination are mouse poliomyelitis virus (Theiler's virus), a cardiovirus member of the *Picornaviridae* family, and mouse hepatitis virus (MHV), a coronavirus member of the nidoviruses. In the present article we review nidovirus infections, which provide experimental model systems for viral persistence, neurotropism, demyelination, and motor neuron pathology.

NIDOVIRUSES: EPIDEMIOLOGY

Nidoviruses form a group of pathogenic, enveloped, RNA viruses that infect many species of animals including humans. In 1996, at the International Congress of Virology in Jerusalem, the term "Nidovirales" or "Nidoviruses" was assigned to an order of viruses that includes 2 families of viruses: *Coronaviridae*, and *Arteriviridae*. The family of *Coronaviridae* includes the coronavirus genus and the torovirus genus. Human coronaviruses (HCV OC43 and 229E) are ubiquitous respiratory and enteric viruses which account for about 15% to 20% of all common colds (4). Mouse hepatitis viruses (MHV) are a group of hepatoencephalitic coronaviruses that are common natural pathogens of mice (5–7). Other members of the coronavirus family include avian infectious bronchitis virus (IBV), bovine coronavirus (BCV), porcine hemagglutinating encephalomyelitis virus (HEV), turkey coronavirus (TCV), porcine transmissible gastroenteritis virus (TGEV), feline coronavirus (FCV), canine coronavirus (CCV), and porcine epidemic diarrhea virus (PEDV) (8).

The family of arterivirus includes the mouse lactate dehydrogenase elevating virus (LDV), porcine reproductive and respiratory syndrome virus (PRRSV), equine arteritis virus (EAV), and simian hemorrhagic fever virus (SHFV) (9). The outcome of arterivirus infection can

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Supported in part by a grant from the National Multiple Sclerosis Society (RG-2615) and a PHS grant (NS30606).

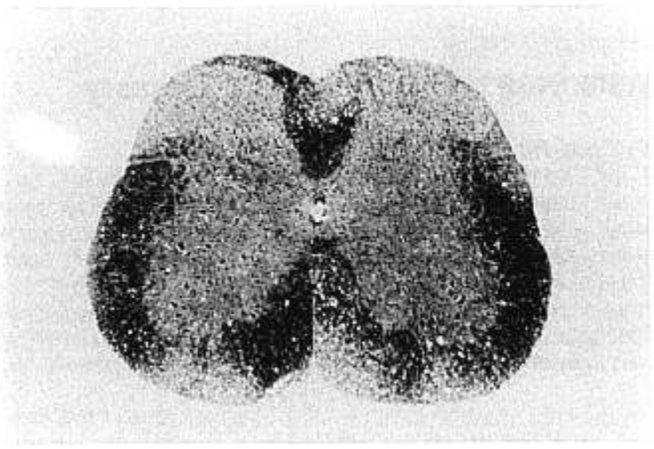

Fig. 1. Coronavirus-induced demyelination. Spinal cord section from a C57Bl/6 mouse 30 days after intracerebral injection with a neurotropic strain (MHV-A59). Note multiple white matter demyelinating plaques (Luxol Fast Blue and Cresyl Violet ×40).

range from an asymptomatic, persistent carrier state to abortion or lethal hemorrhagic fever.

The family of toroviruses includes viruses that are either asymptomatic (10), or cause enteric, respiratory, CNS, and perhaps generalized infection in animals and humans (11). Human toroviruses cause gastroenteritis in children and adults (12–14). Torovirus antibodies or viral particles are found in 90% to 95% of random cattle serum samples and in 81% of swine (15–21). Thus, toroviruses are extremely common pathogens in agriculturally important animals. Toroviruses include equine torovirus (ETV), originally named Berne virus (BEV) (22), and bovine torovirus (BoTV) formerly called Breda virus (BRV), both are gastrointestinal (GI) strains that cause watery diarrhea in their respective hosts (23). In addition, bovine respiratory torovirus (BRTV), a respiratory strain, causes laryngitis, tracheitis and pneumonia in calves (24, 25).

THE NIDOVIRUS GENOME AND VIRAL PARTICLE

The genome of all nidoviruses is a single stranded, linear, positive sense, RNA, measuring 20–32kb (coronaviruses), 25–30kb (toroviruses) and 15kb (arteriviruses) in size. It has a 5′-terminal cap and a 3′-terminal poly(A) tract. The MHV genome is the largest viral RNA genome (26–28). The RNA genome of MHV contains at least 7 genes, termed 1 through 7 encoding 5 structural proteins and 4 or more nonstructural proteins (7, 26, 29). A leader RNA sequence at the 5′-end of the genome may regulate the transcription of MHV-RNAs (29, 30). Gene 1 encodes a set of polyproteins with polymerase functions. Gene 2 encodes 2 proteins including a 30 kD product of ORF 2a (31, 32). Gene 2 also encodes the 65 kD hemagglutinating-esterase (HE) protein in some JHM isolates (33). Genes 3, 6, 7, encoding for S, M, N respectively. Gene 4 encodes a 12–14 kD product (34, 35), and gene 5 encodes a 13 kD product of still unidentified nonstructural protein (34, 36), and the small membrane E protein (37).

The torovirus 5′ two thirds of the genome is occupied by 2 large overlapping open reading frames, ORF1a and ORF1b. These encode a polyprotein from which the viral polymerase is derived. Downstream are 4 smaller open reading frames that are expressed through a 3′-coterminal nested set of mRNAs. They code for the following structural proteins: a 180K precursor of spike protein S; a 26K triple spanning integral membrane protein M; a 65K class I membrane protein (HE) exhibiting acetylesterase activity; and a 19K nucleocapsid protein N (25, 38–42).

The spike (S) glycoprotein is considered the most important part of the virus for its biologic properties, including pathogenesis. Sequence analysis of the MHV S gene revealed a signal sequence at the N-terminal region followed by a receptor-binding domain of approximately 300 aa, which controls attachment of virus to cell receptors. The S protein is comprised of 2 noncovalently associated subunits S1 and S2, which are cleaved by a cellular furin-like enzyme following a cleavage sequence of RRAHR. The S1 subunit forms the globular head in the mature protein and the S2 subunit forms the stalk-like structure with a C-terminal region containing a highly hydrophobic region of a membrane-anchoring domain. The S1 also contains a "hypervariable" region that varies in size among MHV strains and may be associated with pathogenic properties (43, 44). The S2 unit is highly conserved, containing an internal fusion peptide, and 2 heptad repeat domains, thought to be responsible for oligomerization. The S protein plays a major role in fusion of infected cells from within at neutral pH, forming syncytia. Syncytia formation is associated with fragmentation and rearrangement of the Golgi apparatus (45). Host-dependent proteolytic cleavage of S forms two 90 kD units and is an essential step for cell fusion and may be important for virulence (46). The S protein contains determinants of pathogenesis. Mutants that are neutralization resistant following treatment with an anti-S monoclonal antibody are attenuated in vivo and less tropic for neurons (43, 47, 48). Some have point mutation or deletions in the S hypervariable region (43). Pretreatment of mice with some neutralizing anti-S monoclonal antibodies prevented acute disease but allowed the development of chronic demyelination (49). Recent studies using targeted RNA recombination methods show that the S gene contains determinants of neurovirulence and demyelination (50, 51). The exchange of S gene between a nondemyelinating virus (MHV-2) back into a demyelinating virus (A59) background produced recombinant viruses with a

nondemyelinating phenotype (51). The nature of the genomic sites controlling these properties is currently under investigation.

The size of the nidovirus virions varies from the small arterivirus particle to the large particle of coronavirus. The coronavirus virion is an 80–220 nm, enveloped, round but somewhat pleomorphic particle. The torovirus virion is 120–140 nm in diameter, and disk, kidney, or rod shaped. The arterivirus virions are 50–70 nm in diameter and consist of an isometric shape surrounded by a lipid envelope decorated with 12–15 nm ring-like surface structures.

The virions of coronaviruses contain a nucleocapsid phosphoprotein, complexed with the genome RNA form the helical core of the particle. The envelope is composed of a lipid bi-layer and some of the surface proteins are heavily glycosylated. There is a large surface spike or peplomer glycoprotein that gives coronaviruses the typical crown-like appearance in negative stained electron micrographs (52). In addition the envelope also contains an intermediate membrane protein M and a small membrane protein E (37). Some strains also contain a hemagglutinating-esterase protein (HE) (33). The torovirus particle contains similar structural proteins: N, M, S, HE (53). The torovirus M protein may play a role in the intracellular budding process (56). The N protein is a phosphorylated protein with RNA-binding properties (40, 54, 55). The S protein is derived from a 200-kDa precursor (57). Extensive N-glycosylation and proteolytic cleavage of the precursor are part of the post-translational processing of the torovirus S protein. Toroviruses HE protein may play a role in viral adherence to the intestinal wall through the specific, yet reversible, binding to mucopolysaccharides (25). The virions of arteriviruses contain a nucleocapsid protein (N 14k), a nonglycosylated membrane spanning protein (M 16k), and small and large glycosylated surface proteins (GS 25K, GL 30k–42k).

NIDOVIRUS REPLICATION

During lytic infection, virus enters the cell by receptor-mediated viropexis. The receptor molecules for MHV-A59 have been identified as members of the carcinoembryonic (CEA) family of glycoproteins in the immunoglobulin superfamily (58–60). However, the distribution of the receptor molecule in susceptible tissues is not fully known. Infection with LDV appears to have a restrictive entry of virus into permissive cells such as macrophages by a trypsin-sensitive, receptor-mediated, endocytosis (61). A specific receptor on rat macrophages for the enzyme LDH has been demonstrated. It is possible that an analogous receptor exists in mice and may function as the LDV receptor on macrophages (62). The possible role of the MHC class II Ia antigen in LDV-macrophage attachment is controversial (63). The receptors for toroviruses are still unknown.

The genome of nidoviruses acts as a messenger RNA for the synthesis of RNA-dependent RNA polymerase. When translated, the polymerase components are responsible for the formation of a full-length complementary RNA species and for the production of subgenomic mRNA. One species of genomic full length complementary RNA acts as a template for the synthesis of a 3′coterminal nested set of 5 to 7 major subgenomic mRNAs (varies with the virus), that are 5′-capped and 3′-polyadenylated (64). Each mRNA contains the same "leader" sequence in its 5′ end. All mRNAs share a common 3′ end and extend for different lengths in the 5′ direction (65–70). Only the 5′-unique regions of the mRNA are translationally active. Synthesis of mRNA occurs by a unique discontinuous transcription mechanism. A free leader RNA serves as a primer for subgenomic mRNA transcription (71). Further investigations also suggested that coronavirus mRNA synthesis may also occur via transcription from multiple subgenomic negative strand templates (72, 73).

The nidovirus virions are assembled by budding into intracellular membranes of the RER and Golgi apparatus. The incomplete virions then reach the cell membrane through the secretory pathway, where the S protein is added to the envelope and virions are released from the cells (52).

A unique aspect of MHV biology is the high frequency of RNA-RNA recombination between MHVs (74–76). RNA recombination may be important for viral evolution and may contribute to viral pathogenesis. Furthermore, it also provides a useful tool for the study of genetic control of the biological properties of viruses.

NIDOVIRUS PATHOGENESIS

Nidoviruses of both families are naturally transmitted among animals by either the respiratory or enteric routes, or both. Upon entry into the host, nidoviruses may spread into other susceptible tissues and organs, especially the liver, blood vessels, or the CNS. Several biologic properties are common in many nidovirus infections. These properties are especially prominent and may be a prerequisite for the development of neurotropic infections. These include viral persistence, the ability to infect cells of the resident CNS immune system (macrophage-microglial cells or astrocytes), and some suppressive effect on the host immune system.

A variety of DNA and RNA viruses are capable of establishing a long-term existence within the host cell through viral latency or persistence. It is generally accepted that viral latency allows a quiescent existence with limited or no transcription and translation and no production of viral particles. RNA viruses usually persist by evading the immune system and allowing a continuous transcription and translation at a very low level. A large

number of nidoviruses of both the coronavirus and arterivirus families establish persistent infection. However, there is little known about the ability of toroviruses to establish persistent infections. The importance of viral persistence to chronic neurologic disease has been postulated, but a direct cause and effect relationship has not been proven. Moreover, the presence of viral persistence does not guarantee CNS involvement as seen in the case of SHFV, suggesting that additional factors are needed for the development of a nidovirus-induced neurotropic infection.

Coronaviruses produce enteric, hepatic, respiratory, and CNS infections. Some strains of coronavirus MHV are purely hepatotropic (e.g. MHV-2) (77), some are primarily neurotropic (e.g. JHM) (78), while others (MHV-A59, MHV-S, and MHV-3) are both hepatotropic and neurotropic (79–81). Infection of mice with MHV has been extensively used as a model system for viral persistence and for acute and chronic neurologic diseases (5, 49, 79, 82–87). During acute infection with MHV, altered cellular immune functions have been reported, which may correlate with the ability of the virus to infect several immune cells (88). Some of the coronaviruses produce a bi-phasic disease. Acute meningoencephalitis (with or without hepatitis) is the major pathologic process in the first 2 weeks following inoculation with MHV. Subsequently, subacute and chronic diseases develop, which can be divided into 2 major categories: inflammatory demyelinating disease (in JHM and A59) or vasculitis (in MHV-3).

Toroviruses produce both gastrointestinal and respiratory tract infections. There is very little evidence to suggest viral persistence or affinity for the CNS. Toroviruses infect epithelial cells causing villous fusion, atrophy and epithelial desquamation in the small intestine, as well as areas of necrosis in the large intestine (20, 89). Lymphocytic depletion of the Peyer's patches, and activated macrophages associated with edema of the lamina propria are seen (89, 90).

Arterivirus infections cause a variety of diseases. Infection in mice by LDV causes acute poliomyelitis like disease. Increased blood levels of lactate dehydrogenase result in LDV-infected mice by the lytic infection of macrophages (91). Usually this lifelong persistent infection is asymptomatic, except for subtle effects on the host immune system, but it causes a fatal motor neuron disease in certain mouse strains. This virus is also a common contaminant of transplantable mouse tumors (92). Equine arteritis virus causes necrotizing arteritis affecting the media of the small muscle arteries. Infection of EAV in horses ranges from a sub-clinical infection to a systemic influenza-like illness, abortion, and interstitial pneumonia in pregnant horses. Initial viral replication occurs in macrophages (93) with secondary sites of replication in arterial media and endothelial cells. Late term reproductive failure and interstitial pneumonia in pregnant pigs characterize PRRSV infection. The severity of the disease varies greatly depending on the strain of the virus and the age of the host. Infection with SHFV causes a fatal hemorrhagic disease in macaque monkeys. In contrast, patas monkeys that are acutely or persistently infected with SHFV are asymptomatic. Macrophages appear to be the primary target cell for arterivirus replication (94, 95).

Coronaviruses: Viral Persistence Neurotropism and Demyelination

Mouse hepatitis virus is the most studied neurotropic strain of coronaviruses. It produces meningitis, encephalitis (MHV-A59 and JHM), and cerebral vasculitis (MHV-3). Other coronaviruses, such as HEV and BCV, produce meningo-encephalitis in their respective hosts, but their neurotropic properties were not extensively studied.

MHV-induced Meningitis, Encephalitis and Chronic Demyelination: By intranasal or intracerebral inoculation of mice JHM causes panencephalitis, involving the telencephalon, diencephalon, brain stem, cerebellum, and spinal cord. MHV-A59 and certain mutants of JHM produce an anatomically restricted CNS disease. Involved areas include the olfactory and limbic systems and certain basal nuclei and brain stem structures. By tracing the temporal spread of the virus, we (and others) suggested interneuronal transport as the mode of spread within the neuronal cells during acute encephalitis (96–98). Replication of MHV occurs in most CNS cells including neurons, astrocytes, oligodendrocytes, microglia, and endothelial cells. Microglial and EC infection is enhanced in the absence of CD8(+) T cells. Both JHM and A59 cause subacute and chronic inflammatory demyelination in the brain and spinal cord. Propagation of virus from the initial site of infection in the brain to the spinal cord occurs by transport of the virus in neurons and astrocytes (99). Astrocytes in particular may play an important role in this process by secreting cytokines and producing iNOS (100). Perivascular mononuclear (lymphocytic/macrophage) inflammatory infiltration of meninges and the Virchow-Robin spaces is seen adjacent to areas of destruction of myelin and denuded but otherwise intact axons. Macrophages containing myelin debris infiltrate white matter areas, especially in the spinal cord of infected animals (82, 86, 101, 102). Recurrent demyelination, remyelination, regeneration of oligodendrocytes, and increased myelin basic protein gene expression have been demonstrated in various MHV model systems (103–105). These features parallel many of the pathologic findings seen in MS in contrast to the monophasic viral or post viral human demyelinating

diseases such as acute disseminated encephalomyelitis (ADEM) and progressive multifocal leukoencephalopathy (PML).

The mechanism of MHV induced demyelination is not completely understood. Acute encephalitis involves a lytic infection of cells including oligodendrocytes (101). This finding raised the speculation that demyelination may also be caused by direct cytolytic effect of the virus on oligodendrocytes. Several laboratories, including our own, showed evidence of persistent coronavirus infection in both glial cell cultures and in animals. Persistent virions were demonstrated in chronic infection with a temperature sensitive mutant of JHM (106), and persistence of viral genome was found following infection with MHV-A59 (107). Persistent infection of glial cultures with MHV-A59 was used to demonstrate induction of MHC class I expression on astrocytes and oligodendrocytes, mediated by a soluble factor (108–110). MHC class II induction mediated by viral particles has been demonstrated in glial cell infection with JHM (111). The role of an immune mediated pathogenesis in MHV-induced chronic demyelinating disease has also been suggested based on indirect evidence. Adoptive transfer of demyelination with T cells from JHM-infected rats and in-vitro sensitivity to myelin basic protein suggested the possibility that MHV induced demyelination can be at least in part an immune-mediated, EAE-like disease (112). Immunosuppression of mice infected with JHM decreased the incidence of demyelination suggesting that the chronic demyelinating disease is immune-mediated (113). During chronic persistent infection with MHV, mutant viruses with changes in the CTL epitopes develop, suggesting that CTL-escape mutations may play a role in the development of chronic persistent stage (114). Spike deletion mutations may also play a role in the ability of the virus to develop chronic persistence (115).

Arteriviruses: Viral Persistence and Neurotropism

Arteriviruses can cause a persistent infection, which may last from 2 to 3 months in PRRSV infection to a lifetime in LDV, EAV, and SHFV (116). Three of the arteriviruses (LDV, PRRSV and EAV) also produce neurologic diseases. Infection with LDV causes a motor neuron-like poliomyelitis and chronic white matter disease, PRRSV causes meningo-encephalitis, and EAV causes cerebral vasculitis. SHFV has not been associated with a neurologic disease.

LDV-induced Poliomyelitis and Chronic White Matter Disease: Infection with LDV results in a lifelong, asymptomatic, low level viremia. The level of persistent viremia seems to reflect a balance between the production of LDV by infected macrophages and inactivation of the virus in the circulation (62). Viral persistence is maintained by the continuous rounds of cytocidal replication of the virus in new macrophages (117), and is limited by the low rate generation of new permissive macrophages, apparently from nonpermissive precursor cells. The ability to replicate in macrophages may be the primary property of a virus, which allows it to evade host immune defenses and to establish persistence (118), as macrophages normally play a role in the defense against virus infection. Persistence is associated with poorly understood effects on host immunity, including depressed cell mediated immunity and inefficient humoral immunity (119, 120) against resistant LDV isolates selected during the infectious process (121). Moreover, sequestration of virus in infectious virion-antibody complexes (62) enhance efficiency of infection in cultured macrophages (122). The production of autoantibodies may also play a significant role in persistent infection (123).

Infection with LDV produces an age dependant poliomyelitis (ADPM) in mice, a fatal paralytic disease similar to amyotrophic lateral sclerosis of humans. The ADPM disease is produced in immunosuppressed C58 mice, but not in comparable mice of many other strains. Paralytic signs develop 2 to 3 weeks postinfection with a neuropathogenic strain of LDV. Histologically, ADPM is associated with infiltration by inflammatory cells and with neuron destruction in the gray matter of the spinal cord and brain stem. In younger immunosuppressed C58 mice LDV infection may result in histological polioencephalomyelitis without paralytic signs (121, 124). Neuronal damage in ADPE may be directly related to the replication of LDV in these cells. The development of paralysis correlates with the presence of LDV RNA, antigens, and mature virions in motor neurons. The virus probably spreads via axonal transport to cause neuronal destruction without demyelination (125, 126). However, acute encephalomyeloradiculitis with inflammatory lesions in the white matter developed after peripheral inoculation of LDV in 4-to-6-week-old C57BR/cd mice, whether or not the mice were immunosuppressed and regardless of age or sex (121).

The induction of poliomyelitis in mice by LDV is dependent on multiple factors including old age, loss of immune competence, acute infection by LDV, and genetic predisposition. The genetic susceptibility of mice to ADPM is linked to the presence of ecotropic, N-tropic murine leukemia virus (MuLV) provirus, and the $Fv1^{n/n}$ genotype, which permits expression of the virus. Expression of the ecotropic MuLV in glial cells in the spinal cord renders anterior horn neurons susceptible to cytocidal infection by LDV (127). Direct interaction, at the cellular level, between the viruses may cause the fatal paralytic disease. The enhanced susceptibility of C58 mice to ADPM with increasing age and X-irradiation seems to be mediated through an increased expression of the ecotropic MuLV in spinal cord motor neurons (128).

Neuropathogenic and non-neuropathogenic isolates of LDV differ in their ability to infect anterior horn neurons

of immunosuppressed C58 and AKR mice and cause paralytic disease. The non-neuropathogenic isolates are highly resistant to in vivo neutralization by antibodies. They efficiently establish a persistently viremic infection in mice despite a detectable immune response. The neuropathogenic isolates are much more sensitive to antibody neutralization. Thus, paralytic disease is observed only in mice in which the motor neuron protective anti-LDV immune response is suppressed artificially by cyclophosphamide, genetically, or naturally as a result of old age. Under these conditions mice also have an impaired ability to establish a high viremia, persistent infection, in immune competent mice. These properties seem to be interdependent and correlate with the number of N-glycosylation sites on the primary envelope glycoprotein, VP-3P. This glycoprotein is part of the attachment site for the LDV receptor on the permissive cells and harbors an epitope reacting with neutralizing antibodies. The neutralization epitope in the ectodomain of VP-3P may be well masked by the 3 closely spaced polylactosaminoglycan chains present in non-neuropathogenic viruses, leading to their resistance to Ab neutralization. The lack of 2 N-terminal chains in neuropathogenic viruses may render the neutralization epitope less protected, making these viruses more susceptible to suppression by host immune response. In addition, the lack of the 2 chains in neuropathogenic viruses may endow these viruses with the ability to interact with a receptor on anterior horn neurons resulting in neuropathogenesis (129).

PRRSV-induced Meningo-Encephalitis: Infection with PRRSV causes mild, usually asymptomatic encephalitis (130). Occasionally severe meningoencephalitis occurs in neonatal pigs, producing lethargy. In the latter, multifocal lesions are found in the meninges, cerebrum, cerebellum, white periventricular matter, choroid plexus, and perivascular spaces. The lesions contain macrophages, lymphocytes, and a few eosinophils. The cerebellar and cerebral white matter exhibit foci of inflammation and microcystic gliosis. Antigen or RNA of PRRSV is identified in the brain lesions of affected pigs, mainly within cells that are consistent with macrophages or monocytes (131). PRRSV replicates in microglial cells in vitro, thus demonstrating its potential for replication in the brain. Differences in virus envelope glycoproteins or envelope gene sequence may be related to the differences in neurovirulence.

Infection of swine with PRRSV has the capacity to persist for several months. The first 2 weeks of infection are considered as the acute stage, during which maximal virus titers are recovered from all susceptible organs. The persistent stage follows with lower levels of virus replication in some organs (132) and may last for several months (9). Competent immune response does occur after PRRSV infection, however persistent infection indicates the ineffectiveness of the humoral and the cellular immune response in some pigs (133). Moreover the circulating antibody may even enhance virus uptake by macrophages through Fc receptors.

EAV-induced Cerebral Arteritis: Brain sections of aborted fetuses exhibit disseminated inflammatory lesions and a few perivascular mononuclear inflammatory infiltrates in the cerebrum and anterior midbrain. Infectious virus is isolated from the fetuses brains (134). Persistent EAV is observed in renal tissue of experimentally infected horses (93). However, it is unclear whether persistent renal lesions are caused by EAV replication in these tissues or are the result of the accumulation of complement fixing antibody-virus complexes. A more definitive chronic carrier state has been identified in stallions. Sixty percent of stallions infected with EVA become persistently infected (135) for a lifetime. Little is known about the mechanism of EAV persistent infection and how the virus evades host defense mechanism.

SUMMARY

Nidoviruses of both families of viruses contain common biologic and molecular features including a similar genomic organization and replication strategy. The common pathogenic features of CNS infections of nidoviruses include viral persistence, affinity for resident CNS immune cells such as macrophage/microglia and astrocytes, and cellular and humoral immune system impairment. These common features allow us to propose the following model for nidovirus pathogenesis. Nidoviruses develop low level persistent infection by suppressing functions of the immune system that are required for viral clearance. When motor neurons are cell targets in the CNS, persistent infection may be established in these cells, especially if this is facilitated by coinfection with an ecotropic retrovirus as in LDV infection. The suppressive effect on one arm of the immune system may induce another arm of the immune system to initiate an autoimmune phenomenon against infected oligodendrocytes and/or myelin components (as in MHV infection). In addition, the affinity of the virus for microglia and/or astrocytes, as in MHV infection, allows a persistent infection in these cells to cause alterations in the local CNS immunologic environment, including alteration in cytokine secretion profile and the promotion of apoptosis. A detailed analysis of the correlation between the molecular structure of nidoviruses and the mechanisms of neurotropism and CNS disease awaits further investigation.

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