

A Review of Coronavirus Infection in the Central Nervous System of Cats and Mice

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Feline infectious peritonitis (FIP) is a common cause of death in cats. Management of this disease has been hampered by difficulties identifying the infection and determining the immunological status of affected cats and by high variability in the clinical, pathological, and immunological characteristics of affected cats. Neurological FIP, which is much more homogeneous than systemic effusive or noneffusive FIP, appears to be a good model for establishing the basic features of FIP immunopathogenesis. Very little information is available about the immunopathogenesis of neurologic FIP, and it is reasonable to use research from the well-characterized mouse hepatitis virus (MHV) immune-mediated encephalitis system, as a template for FIP investigation, and to contrast findings from the MHV model with those of FIP. It is expected that the immunopathogenic mechanisms will have important similarities. Such comparative research may lead to better understanding of FIP immunopathogenesis and rational prospects for management of this frustrating disease.

Key words: Cats; Feline infectious peritonitis; Mouse hepatitis virus; Neurological disease.

Feline infectious peritonitis (FIP) is a fatal, immune-mediated disease produced as a result of infection of macrophages by mutant feline coronavirus strains (FIPVs). The severity of FIP is determined by virus strain and by host-specific, partially heritable immune responses.¹ The causative agent, FIP virus (FIPV), is a macrophage-tropic mutant of the ubiquitous feline enteric coronavirus (FECV).^{2–4} These feline coronaviruses are closely related to transmissible gastroenteritis virus (TGEV) of pigs and more distantly related to MHV (a coronavirus of rodents), human respiratory coronavirus, and others. As with most RNA viruses, a high rate of point or other small-scale mutations and larger scale recombination events occur. Type II FECVs are viruses that arise as a result of recombinations between type I FECV and canine coronavirus (CCV). Type II FECVs acquired a canine S gene and express a canine S gene product.⁵ Mutations are common in the 7b open reading frame (ORF) of both type I and type II FECVs and may be associated with reduced virulence.^{4,6} The 7b ORF arose in the FECV/CCV lineage and encodes a nonstructural secretory glycoprotein of undetermined function, which is not necessary for viral replication.⁷ Deletions, point mutations, and frame-shift mutations leading to early truncation of the 3c ORF also are common.^{4,6} Because FIP-defining mutations may occur in the S, 3c, and 7b genes, polymerase chain reaction (PCR) with these genes as a target cannot discriminate between benign FECV and fatal FIPV.^{2–4}

Murine hepatitis virus shares most genes with the FECVs, including M (membrane glycoprotein), E (small

membrane), N (nucleocapsid), and S (spike glycoprotein), which is post-translationally modified to S1 and S2.⁸ The MHV genome, however, also codes for an HE protein and does not contain a 7b ORF. Mutations in the E and S proteins lead to attenuation of MHV.⁹ A third coronavirus, HCV-229E, has been implicated in neurological disease in people.¹⁰

Clinical Manifestations of Coronaviral Infection in the Central Nervous System (CNS)

FIPV in Cats

FIP occurs most frequently in cats younger than 3 years of age from multiple-cat homes (shelters and breeding catteries).^{11,12} One-quarter to one-third of cats with noneffusive FIP have either primary neurological FIP or neurological abnormalities as a part of their overall disease presentation (Foley and Pedersen, unpublished data).¹³ The immunological and pathological characteristics of neurological FIP, however, are much more stereotypic than those of systemic FIP. Thus, neurological FIP may be useful for studying basic mechanisms of FIP pathogenesis. The extent to which genetic differences among viral strains confer relative neurotropism is unknown, but some cats with severe neurological disease have mild or undetectable systemic disease.

Both neurological and generalized FIP may present first as a nonspecific illness, with clinical signs including weight loss, weakness, fever, and lethargy. Abdominal abnormalities are detected commonly on physical examination of cats with neurological FIP, including mesenteric lymphadenopathy and irregular splenic and renal surfaces.^{14,15} Common historical findings in cats with neurological FIP include dementia, pica, seizures, inappropriate elimination, incontinence (fecal and urinary), and compulsive licking.^{15,16} Neurological examination may identify ataxia, hyperesthesia, reduced consciousness, hyperreflexia, crossed-extensor reflexes, reduced conscious proprioception, caudal paresis, cerebellar-vestibular signs, or cranial nerve deficits.^{14,15,17–19} Ophthalmic lesions also are common in neurological FIP, including anterior uveitis, keratic precipitates, flocculent debris in the anterior chamber, retinitis, and anisocoria.¹⁵

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MHV in Mice and Rats

Murine hepatitis virus, like FIPV, can produce either systemic infection or disease primarily affecting the liver. Well-defined neurotropic genetic variants of MHV also occur, including a well-studied variant designated MHV-JHM, which is responsible for progressive encephalomyelitis and death in infected mice and rats. Intranasal or intracranial inoculation of MHV-JHM in BALB/C or C57Bl/6 mice leads to rapid, fatal encephalitis.²⁰ The syndrome in survivors (either because the virus is attenuated or the host is resistant, vaccinated, or a pup from a vaccinated dam) consists of chronic demyelination and hindlimb paralysis²¹ and has been proposed as a model for multiple sclerosis.²² Determinants of neurovirulence are found in the MHV S gene.^{23,24} A second variant of MHV, MHV-OBLV60, leads to neuronal infection specifically in the anterior olfactory bulb after intranasal challenge in mice.²⁵

Pathology of Coronavirus-Associated Neurological Disease

FIP Pathology

The definitive lesion of FIP is a pyogranuloma that results from immune-mediated phenomena secondary to coronaviral infection of macrophages. The most common sites are serosal, pleural, meningeal, ependymal, or uveal membranes. In the earliest stages of abdominal FIP, diffuse alterations with activated mesothelial cells and a few coronavirus-infected macrophages or an exudative precipitate may be detected on serosal surfaces.²⁶ Larger pyogranulomas become grossly visible, ranging from small lesions, often on the renal capsular surface, to severe, generalized miliary granulomatous lesions that distort renal surfaces, disseminate throughout the omentum and gastrointestinal serosa, and invade splenic and hepatic parenchyma. Gross lesions of FIP in the CNS may be subtle, with ependymitis, thickening and opacification of meninges, and ventricular dilatation, usually in the fourth ventricle and least often in the lateral ventricles.¹⁵ Lesions occur most commonly on the ventral surface of the brain, often accompanied by secondary obstructive hydrocephalus.

Histopathologically, lesions in the brains of cats with FIP consist of meningitis, ependymitis (ranging from mild ependymal infiltration to complete effacement of the ependymal lining by a heavy infiltrate of histiocytes and lymphocytes), periventriculitis, and choroiditis of varying severity, often superficial and oriented around the ventricles, with dense infiltrates of lymphocytes, plasma cells, neutrophils, and macrophages.^{14,15,27-31} Meningitis may be more severe on the ventrocaudal surfaces of the brain, especially at the base of the cerebellum and the brainstem, including the medulla oblongata. In the meninges, the inflammatory cells may have a predominantly perivascular distribution, forming cuffs around arteries (periarthritis) and infiltrating the wall of veins and venules (phlebitis), with exudation of a cell- and protein-rich edema fluid, and periventricular reactive astrocytosis. The inflammation may extend into the superficial neuropil, as well as into cranial nerve roots.¹⁵ If lesions are deep, they usually are perivascular with scattered glial nodules.²⁷ Hydrocephalus is seen in association

with leptomeningitis, meningeal fibrosis, accumulation of cellular debris, and obstruction of the cerebrospinal fluid flow.

Pathology of Neurotropic MHV

Pathologic lesions after MHV infection in the brains of rats and mice are variable, depending on viral dose and route of administration and rodent genotype, age, and immunological characteristics.³² In severe acute JHM encephalitis, necrotizing lesions are found in the gray and white matter, with axonal changes, including disintegration of neurofilaments.³² Within the necrotic areas, perivascular neutrophilic infiltrates occur especially in the subependyma, choroid, and meninges. In acute MHV-A59 infection in CD8+ T-cell deficient mice, periventricular encephalitis occurs with lymphocytic infiltration into the choroid plexus, ependyma, and subependymal brain tissue.³³ Lesions in MHV-OBLV60 consist of local neuronal infection, some mitral cell destruction, and T-cell inflammation with astrocytosis.²⁵ Some evidence suggests a pathological component of vasculitis, with mouse endothelial cell lines susceptible to MHV-JHM exhibiting cytopathology within several days of infection.³⁴

The subacute to chronic immune-modulated pathological changes that occur with manipulation of the MHV-JHM system are particularly relevant to the murine model of FIP. Depending on mouse strain and immunological status, MHV-JHM produces meningeal inflammation associated with T-cells and macrophages and demyelination but relatively little disease in axons. Demyelination occurs within and adjacent to areas of inflammation and astrocytic proliferation.^{32,35} Neutrophils and monocytes have been observed infiltrating through endothelial cell junctions and participating in the phagocytosis of myelin debris.³² Perivascular cuffing by macrophages is observed in chronic encephalitis, as in FIP.

Neurotropic Coronavirus Entry into the CNS and Cell Tropism

FIPV Access and Persistence in the CNS

The route of entry of FIPV into the CNS is unknown. The FIPV virus probably travels hematogenously in macrophages, and 1 study reported a cat with positive immunohistochemical staining for FIPV in monocytes in blood vessels of the choroid plexus.³⁶ Once in the CNS, there is little evidence that FIPV enters any cells other than macrophages. Foley et al¹⁵ reported positive immunohistochemical staining (by means of a mouse monoclonal antibody against the FIPV N protein) for FIPV, primarily in macrophages in FIP granulomas but also in some lymphocytes. Virus-infected cells were numerous in some areas of intense ependymitis and choroiditis and free within the ventricular lumen, with very few positive cells in the meningeal infiltrates. No staining was observed in vascular basement membranes or in cells of the neuropil. Macrophages in necrotic regions and in the center of lesions often are not infected with coronavirus.³⁷

Neurotropic MHV Entry into the CNS

Neurotropic MHV strains have several routes of entry into the CNS. Some studies show that MHV-JHM travels up the olfactory nerve and enters the CNS.³⁸ This is not surprising, because coronaviruses generally are epitheliotropic, and neurons share embryological origin with epithelial cells. CNS infection with JHM also may occur after peripheral infection and viremia.^{39,40} Receptors for neurotropic human coronavirus HCV-229E have been detected not only in human lung cells but also in human neuron, astrocyte, and oligodendrocyte cell cultures.⁴¹ HCV-229E also infects macrophages and endothelial cells, however, suggesting hematogenous introduction into the CNS,^{40,42} similar to FIP.

Once inside the CNS, the major targets for MHV are glial cells and neurons.³⁵ Especially in neuroattenuated strains, MHV-JHM infects primarily oligodendrocytes⁴³ and has been reported in astrocytes.⁴⁴ MHV-4 infects mainly neurons.²⁰ Subsequent events in chronic disease pathogenesis include recruitment of inflammatory cells and the interactions of immune cells with the virus-infected cells. Macrophages, activated T-cells, and some B-cells may cross into the intact CNS through the blood-brain barrier, with the potential for major cytokine upregulation.^{45,46}

The Immunopathogenesis of Neurologic Coronavirus Infections

FIP Immunopathogenesis

After establishment of FIPV in the CNS, mechanisms of disease are primarily immune-mediated, involving humoral and cell-mediated immunity (CMI). Coronavirus-infected macrophages can trigger massive complement activation and deposition of C3 on affected surfaces, disseminated intravascular coagulopathy, vessel necrosis, and effusion.^{37,47–49} However, immunopathogenic events in neurological FIP have not been well described.

Antibodies in FIP

Both in systemic and neurological FIP, antibodies (especially to the spike protein) contribute to the opsonization of viral antigen and have the capacity to mediate or enhance disease.^{50–54} Antibodies to FECV and FIPV are identical. Anti-FIPV IgG and IgM-producing B-cells are present in FIP lesions, at the interface of healthy tissue and granulomas, and in the serum and CSF of cats with neurological FIP.^{26,37} Apparently, some antibody production occurs locally in the CNS, in response to viral antigen in the brain. In one study, serum coronavirus titers of 16 cats with neurological FIP were positive, with a median titer of 1:400, whereas CSF titers were positive in 15 cats, with a median titer of 1:100.¹⁵ Two cats had high protein concentrations and increased cells, predominantly lymphocytes and neutrophils. The titer in CSF was not statistically correlated with serum titer, and the ratio of serum:CSF titer was not correlated with the serum:CSF protein ratio. If passive leakage of protein from serum into the CSF were invoked to explain the presence of antibodies, it would be expected that the total protein:anti-FIPV IgG ratios would be similar in both serum and CSF. In contrast, CSF IgG titers tended

to be proportionally much higher than serum titers. This finding suggests that anti-FIPV IgG may have been produced in the tissues of the brain in response to a locally replicating virus.

FIP Cell-Mediated Immunity and Cytokine Changes

Although almost nothing is known about the mechanism, cell-mediated immunity has been hypothesized to be protective against FIP.^{50–54} CD4+ T-cells commonly are observed in FIP granulomas.³⁷ During acute experimental multisystemic FIP, apoptosis and T-cell depletion were observed in the spleen and mesenteric lymph nodes.⁵⁵ This effect was induced by heat-treated effusion fluid (presumably containing some cytokines) but not tissue culture fluid.

Likewise, little is known about the induction of cytokines during the course of FIP. Preliminary findings suggest that development of FIP appears to be associated with a switch to predominantly Th2 immunity, with increases in IL-10 concentrations.⁵⁶ Goitsuka et al⁵⁷ described increases in IL-6 and IL-1, but Gunn-Moore et al⁵⁸ reported reductions in TH₂ and TH₁ cytokines including IL-2, IL-4, IL-10, and IL-12, which they attributed to general immunosuppression. Mildly increased amounts of IL-1 α mRNA are detected inconsistently in association with lesions in cells of many organs in cats with FIP.⁵⁹ Roles of IL-1 in this setting may include vascular endothelial activation, regulation of macrophages, IL-8 and IL-6, and chemotaxis. IL-6 and IL-1 can increase B-cell growth and differentiation and could exacerbate humoral contributions to the severity of FIP. The inflammatory cytokines TNF- α , IL-1 α , and IL-6 could circumvent the blood-brain barrier during systemic FIP, or they could cross the blood-brain barrier after reorganization of the endothelial actin cytoskeleton.^{60,61} No information is available regarding cytokine production in neurological FIP, and how cytokines in the CNS compare to those in abdominal tissues of cats with generalized or effusive FIP is unknown.

MHV Immunopathogenesis: Chronic Encephalitis with Demyelination Is an Immune-Mediated Disease

MHV neurological disease can range from severe, acute, necrotizing, rapidly fatal encephalitis to chronic immune-mediated demyelination with little encephalitis, depending on mouse strain, age, and immunocompetence. The appreciation of the principal role of the immune system in producing demyelination emerged from a series of experiments performed over several decades. Profoundly immunosuppressed mice develop high concentrations of virus in the CNS, but demyelination and clinical signs are minimal. Immunocompetent mice have variable or even low concentrations of virus but develop marked disease. Immune reconstitution in immunocompromised mice results in the development of severe demyelinating disease.

If mice are pretreated with passive infusions of antibodies or T-cells or if they receive neuroattenuated MHV strains, they develop chronic, but not fatal, disease after MHV-JHM infection.^{62,63} Immunocompetent C57BL/6 mice clear MHV-JHM virus from the brain but develop severe immune-mediated demyelination and paralysis.²² In contrast, severe combined immunodeficient (SCID) mice have

persistent viral loads but no neurologic impairment or detectable lesions. Gamma-irradiated immunocompromised mice similarly were resistant to chronic demyelination, but reconstitution of the immune system with adoptive transfer of splenocytes restored the immune-mediated lesions.⁶⁴

Cytotoxic T-Lymphocytes (CTLs) and Apoptosis in MHV

Both CD4+ and CD8+ T-cells apparently are required to clear MHV from the CNS. CD8+ T-cells are the predominant infiltrating leukocyte in Lewis rats with MHV-JHM and paralytic disease,⁶⁵ whereas infiltration in clinically normal MHV-JHM-positive brown Norway rats consists of CD4+ cells. CTLs may kill some virally infected cells and protect mice from fatal disease, but they do not completely eliminate virus.⁶⁶ When CD8+-depleted mice are reconstituted, virus load is reduced, and infection is not detected in most infected cell types, except for oligodendroglial cells.⁶⁶ Mice with nucleocapsid or spike protein-specific CD8+ T-cells develop chronic demyelinating disease.^{21,67} In the MHV-OBLV60 model, depletion of CD8+ cells is associated with delayed clearance of OBLV60 (but infected mice did recover).²⁵ CTLs probably exacerbate lesions by contributing to tissue damage. MHV is relatively labile genetically (a common characteristic of RNA viruses), and mutant strains with recognition sites that can evade CTL-mediated viral killing apparently increase and become the predominant viral strains in response to CTL-mediated natural selection. This feature results in disease progression in immunocompetent, but not immunosuppressed, hosts infected with these mutants.⁶⁸

Several studies have suggested that apoptosis may be important in clearing virus from the CNS. Specific CTL recognition promotes apoptosis of MHV-infected cells.⁶⁹ In experimental allergic encephalomyelitis in rats, apoptosis appears to help control inflammation.⁷⁰

CD4+ Cells in MHV

The role of CD4+ cells in MHV infection is also complex. Nucleocapsid or spike protein-specific CD4+ T-cells have been shown to protect mice from coronaviral encephalomyelitis in the absence of CD8+ T-cells.⁷¹ If mice with MHV-OBLV60 had CD8+ but not CD4+ cells, they developed persistent infection. If they were CD8+ deficient, but CD4+ cells were normal, they had delayed clearance of the virus, suggesting a primary role for CD4+ cells in clearing this virus.²⁵ Sussman et al⁷² and Williamson and Stohlman⁷³ documented the requirement for CD4+ cells for clearance of JHM from mice. However, γ -irradiated mice that were reconstituted with CD4+ cells responded to MHV-JHM challenge with earlier and more severe onset of neurological disease.⁷⁴ CD4+ knockout mice had less inflammation (with fewer macrophages and microglial cells) and less demyelination than did CD4+ competent mice.⁷⁵

Cytokine Changes in MHV

The presence of MHV-JHM in astrocytes triggers a cytokine cascade that contributes to demyelination.⁷⁶ Sun et al⁷⁷ documented production of TNF- α , IL-1 β , and IL-6 by

astrocytes in the spinal cords of mice that were chronically infected with MHV-JHM, localized to areas of virus infection and demyelination. TNF- α and IL-6 are also produced in the brains of acutely infected mice, but the major cell producing these cytokines is the macrophage. These cytokines may help recruit T-cells and monocytes and may increase vascular permeability.⁷⁸ IL-1 β promotes leukocyte adhesion to endothelial cells, and TNF- α is toxic to oligodendrocytes.⁶³ The role of IL-6 is unclear. Effects attributed to IL-6 include recruitment and activation of T-cells and macrophages, expansion of CTLs, modulation of plasma cell differentiation, increased vascular permeability, down-regulation of acute phase proteins, and contributions to immune-mediated destruction in the CNS.⁷⁹⁻⁸²

In a study of cytokine profiles in lethally compared to sublethally affected mice with JHM, both TH₁- and TH₂-type cytokines, including IFN- γ , IL-4, and IL-10, were induced in all mice.⁸³ In the mice that died, TNF- α was induced more rapidly, and IL-1 α was increased. In mice with nonlethal infections, IL-12 and IL-1 β were increased, and IL-6 was expressed early. Minor differences were observed in the patterns of inflammation in IL-10-deficient compared to syngeneic mice, but the outcome of infection (eg, mortality and virus load) was not affected.⁸⁴ These results, combined with those of Sun et al,⁷⁷ suggest that IL-1 β may allow mice to survive the early stages of the disease but may not allow for clearance of chronic infection.

Interferon- γ also appears to be important for clearance of MHV infection. IFN- γ -deficient mice developed persistent JHM infection with increased clinical signs and mortality compared to mice competent to produce IFN- γ .⁸⁵ Antiviral antibody and CTL responses were normal in IFN- γ -negative mice, despite the fact that IFN- γ modulates antibody production. Viral antigen occurred in oligodendrocytes and in association with CD8+ T-cells, suggesting that IFN- γ is necessary for control of viral replication in oligodendrocytes. Treatment of mice with anti-IFN- γ antibody increased the mortality rate of mice, whereas immunotherapy with IFN- γ reduced mortality and virus load.⁸⁶

MHV-OBLV60 infection in immunocompetent mice induced transient mRNA upregulation for cytokines IL-1 α , IL-1 β , IL-6, TNF- α , and IFN- γ .²⁵ Nude mice differed in their cytokine profiles in that they lacked mRNA for IFN- γ , whereas concentrations of the other cytokines remained persistently high. The authors suggested that the increased cytokines in the nude mice were produced by CNS glial cells and possibly CD4+ and CD8+ subsets. IFN- γ treatment of human neuronal cell cultures markedly increased the susceptibility of cells to infection with HCV-OC43 (although cells were susceptible to 229E without IFN- γ treatment).⁸⁷ In this study, the hypothesized role of IFN- γ was induction of MHC class I expression. IFN- γ may increase superoxide dismutase and protect against oxygen radicals, kill virus directly, and increase the cytotoxicity of other effector cells.

The chemokine CRG-2 is expressed primarily by astrocytes during MHV infection and co-localizes with areas of viral RNA and demyelination.⁸⁸ The function of CRG-2 is not known, but this chemokine is also found in simian immunodeficiency viral (SIV) encephalitis⁸⁹ and lymphocytic choriomeningitis.⁹⁰

Prospects for Immunomodulation of FIP with Insight from the Mouse Model

With very little information available about the immunopathogenesis of neurologic FIP, research performed in mice can be used as a model for FIP and to contrast findings from the MHV model with those in FIP. The immunopathogenic mechanisms are expected to have important similarities, but the demyelination that occurs in MHV is a more extensive pathological sequela of coronaviral infection (but not more fatal for the patient) than the focal granulomas that occur in FIP. It can be hypothesized that in FIP, upregulation of cytokines IL-1 β , IL-6, IFN- γ , and TNF- α may be important in mediation of destructive inflammation, but cytokines IL-6 and IFN- γ may be important in controlling viral infection, despite adverse inflammatory effects. Cytokine and chemokine alterations in the brains of mice and cats with coronavirus infections may provide valuable clues to the immunopathogenesis of these diseases, as well as possible diagnostic markers and targets for immunological treatment of disease.

It is tempting to consider cytokine modulation in the treatment of FIP, but this application is premature without information regarding the cytokine profiles of affected cats. In addition to characterizing the concentrations of cytokines in the CNS of cats with FIP, it will be important to describe the timing of upregulated cytokine transcription. In mice with HMV-JHM, treatment with recombinant IFN- γ resulted in reduced virus load in the liver but not in the brain.⁸⁶ If specific pro-inflammatory cytokines are found to be consistently high in cats with neurological FIP, use of cytokine antagonists may eventually be of benefit in treatment.

Successful management of FIP may consist of better methods of prevention as well as management of disease once it occurs. A commercial FIP vaccine is available that consists of a mucosally delivered, temperature-sensitive mutant form of FIPV. The vaccine virus is supposed to undergo replication only in outer oronasal cavities at low temperatures, thus triggering protective antibodies but not FIP. A few controversial studies have documented reduction in FIP as a result of vaccination.^{91,92} Many vaccinated cats, however, fail to seroconvert with either IgG or IgA (Foley and Pedersen, unpublished data), and independent studies failed to identify vaccine efficacy.^{93,94} Other vaccines, including DNA vaccines, have not progressed beyond experimental stages either because of lack of seroconversion, lack of protection, or both. In contrast with FIP vaccines, vaccines against MHV have been shown to protect mice from challenge with virulent virus. A vaccine with purified spike protein⁹⁵ and synthetic S2 led to neutralizing antibodies in vivo.^{96,97} Recombinant subunits against S in tobacco mosaic virus were given subcutaneously or intranasally and were protective against MHV-JHM.⁹⁸

The current standard of care for immunomodulation of FIP is immunosuppressive doses of prednisone, to sustain an acceptable quality of life for as long as possible. The effects of steroids include lymphocytolysis, inhibition of arachidonic acid metabolism, reduction in cytokine RNA transcription, and nitric oxide synthesis inhibition.⁹⁹ Other potentially useful anti-inflammatory drugs include antileukocyte antibodies and antioxidants. These treatments are only palliative. A better understanding of the fundamental pathogenesis of FIP will be

necessary to offer better treatment and, ultimately, cure of this disease.

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References

1. Foley J, Pedersen N. The inheritance of susceptibility to feline infectious peritonitis in purebred catteries. *Feline Pract* 1996;24:14–22.
2. Poland AM, Vennema H, Foley JE, et al. Two related strains of feline infectious peritonitis virus isolated from immunocompromised cats infected with a feline enteric coronavirus. *J Clin Microbiol* 1996;34:3180–3184.
3. Vennema H, Poland A, Hawkins KF, et al. A comparison of the genomes of FECVs and FIPVs and what they tell us about the relationships between feline coronaviruses and their evolution. *Feline Pract* 1995;23:40–46.
4. Vennema H, Poland A, Foley J, et al. Feline infectious peritonitis viruses arise by mutation from endemic feline enteric coronaviruses. *Virology* 1998;243:150–157.
5. Herrewegh AA, Smeenk I, Horzinek MC, et al. Feline coronavirus type II strains 79-1683 and 79-1146 originate from a double recombination between feline coronavirus type I and canine coronavirus. *J Virol* 1998;72:4508–4514.
6. Herrewegh AA, Vennema H, Horzinek MC, et al. The molecular genetics of feline coronaviruses: Comparative sequence analysis of the ORF7a/7b transcription unit of different biotypes. *Virology* 1995;212:622–631.
7. Vennema H, Heijnen L, Rottier PJ, et al. A novel glycoprotein of feline infectious peritonitis coronavirus contains a KDEL-like endoplasmic reticulum retention signal. *J Virol* 1992;66:4951–4956.
8. Sturman LS, Ricard CS, Holmes KV. Proteolytic cleavage of the E2 glycoprotein of murine coronavirus: Activation of cell-fusing activity of virions by trypsin and separation of two different 90K cleavage fragments. *J Virol* 1985;56:904–911.
9. Dalziel RG, Lampert PW, Talbot PJ, et al. Site-specific alteration of murine hepatitis virus type 4 peplomer glycoprotein E2 results in reduced neurovirulence. *J Virol* 1986;59:463–471.
10. Jouvenne P, Mounir S, Stewart JN, et al. Sequence analysis of human coronavirus 229E mRNAs 4 and 5: Evidence for polymorphism and homology with myelin basic protein. *Virus Res* 1992;22:125–141.
11. Pedersen NC. An overview of feline enteric coronavirus and infectious peritonitis virus infections. *Feline Pract* 1995;23:7–22.
12. Foley JE, Poland A, Carlson J, et al. Risk factors for feline infectious peritonitis among cats in multiple-cat environments with endemic feline enteric coronavirus. *J Am Vet Med Assoc* 1997;210:1313–1318.
13. Fenner W. Inflammations of the nervous system. In: August J, ed. *Consultations in Feline Internal Medicine*. Philadelphia, PA: WB Saunders; 1991:507–517.
14. Kornegay J. Feline infectious peritonitis: The central nervous system form. *J Am Anim Hosp Assoc* 1978;14:580–584.
15. Foley J, Lapointe J-M, Koblik P, et al. Diagnostic features of neurologic feline infectious peritonitis. *J Vet Intern Med* 1998;12:36–63.
16. Kline K, Joseph R, Averill D. Feline infectious peritonitis with neurological involvement: Clinical and pathological findings in 24 cats. *J Am Anim Hosp Assoc* 1994;30:111–118.
17. Pedersen N. Feline infectious peritonitis and feline enteric coronavirus infections. Part 2: Feline infectious peritonitis. *Feline Pract* 1983;13:5–19.

18. Greene CE, McDermott M, Jameson PH, et al. *Bartonella henselae* infection in cats: Evaluation during primary infection, treatment, and rechallenge infection. *J Clin Microbiol* 1996;34:1682–1685.
19. August J. Feline infectious peritonitis: An immune-mediated coronavirus vasculitis. *Vet Clin North Am Small Anim Pract* 1984;14:971–984.
20. Fazakerley JK, Parker SE, Bloom F, et al. The V5A13.1 envelope glycoprotein deletion mutant of mouse hepatitis virus type-4 is neuroattenuated by its reduced rate of spread in the central nervous system. *Virology* 1992;187:178–188.
21. Perlman S, Schelper R, Bolger E, et al. Late onset, symptomatic, demyelinating encephalomyelitis in mice infected with MHV-JHM in the presence of maternal antibody. *Microb Pathogenesis* 1987;2:185–194.
22. Houtman J, Fleming J. Dissociation of demyelination and viral clearance in congenitally immunodeficient mice infected with murine coronavirus JHM. *Neurovirology* 1996;2:101–110.
23. Buchmeier MJ, Dalziel RG, Koolen MJ, et al. Molecular determinants of CNS virulence of MHV-4. *Adv Exp Med Biol* 1987;218:287–295.
24. Fleming JO, Trousdale MD, Bradbury J, et al. Experimental demyelination induced by coronavirus JHM (MHV-4): Molecular identification of a viral determinant of paralytic disease. *Microb Pathogenesis* 1987;3:9–20.
25. Pearce BD, Hobbs MV, McGraw TS, et al. Cytokine induction during T-cell-mediated clearance of mouse hepatitis virus from neurons in vivo. *J Virol* 1994;68:5483–5495.
26. Kipar A, Bellmann S, Kremendahl J, et al. Cellular composition, coronavirus antigen expression and production of specific antibodies in lesions in feline infectious peritonitis. *Vet Immunol Immunopathol* 1998;65:243–257.
27. Slauson D, Finn J. Meningoencephalitis and panophthalmitis in feline infectious peritonitis. *J Am Vet Med Assoc* 1972;160:729–734.
28. Krum S, Johnson K, Wilson J. Hydrocephalus associated with the non-effusive form of feline infectious peritonitis. *J Am Vet Med Assoc* 1975;167:746–748.
29. Legendre A, Whitenack D. Feline infectious peritonitis with spinal cord involvement in two cats. *J Am Vet Med Assoc* 1975;160:729–734.
30. Summers B, Cummings J, de Lahunta A. Feline infectious peritonitis. In: *Veterinary Neuropathology*. St. Louis, MO: Mosby Year-Book; 1995:119–121.
31. Tamke P, Petersen M, Dietze A, et al. Acquired hydrocephalus and hydromyelia in a cat with feline infectious peritonitis: A case report and brief review. *J Can Vet* 1988;29:997–1000.
32. Lampert PW, Sims JK, Kniazeff AJ. Mechanism of demyelination in JHM virus encephalomyelitis. Electron microscopic studies. *Acta Neuropathol* 1973;24:76–85.
33. Lavi E, Wang Q. The protective role of cytotoxic T cells and interferon against coronavirus invasion of the brain. In: Talbot P, Levy G, eds. *Corona- and Related Viruses*. New York, NY: Plenum Press; 1995:145–149.
34. Cabirac G, Murray R, McLaughlin L, et al. In vitro interaction of coronaviruses with primate and human brain microvascular endothelial cells. In: Talbot P, Levy G, eds. *Corona- and Related Viruses*. New York, NY: Plenum Press; 1995:79–88.
35. Weiner LP. Pathogenesis of demyelination induced by a mouse hepatitis. *Arch Neurol* 1973;28:298–303.
36. Tammer R, Eversen O, Lutz H, et al. Immunohistological demonstration of feline infectious peritonitis virus antigen in paraffin-embedded tissues using feline ascites or murine monoclonal antibodies. *Vet Immunol Immunopathol* 1995;49:177–182.
37. Paltrinieri S, Cammarata MP, Cammarata G, et al. Some aspects of humoral and cellular immunity in naturally occurring feline infectious peritonitis. *Vet Immunol Immunopathol* 1998;65:205–220.
38. Barnett EM, Perlman S. The olfactory nerve and not the trigeminal nerve is the major site of CNS entry for mouse hepatitis virus, strain JHM. *Virology* 1993;194:185–191.
39. Barthold SW, Smith AL. Viremic dissemination of mouse hepatitis virus-JHM following intranasal inoculation of mice. *Arch Virol* 1992;122:35–44.
40. Cabirac GF, Soike KF, Zhang JY, et al. Entry of coronavirus into primate CNS following peripheral infection. *Microb Pathogenesis* 1994;16:349–357.
41. Lachance C, Arbour N, Cashman NR, et al. Involvement of aminopeptidase N (CD13) in infection of human neural cells by human coronavirus 229E. *J Virol* 1998;72:6511–6519.
42. Patterson S, Macnaughton MR. Replication of human respiratory coronavirus strain 229E in human macrophages. *J Gen Virol* 1982;60:307–314.
43. Taguchi F, Siddell SG, Wege H, et al. Characterization of a variant virus selected in rat brains after infection by coronavirus mouse hepatitis virus JHM. *J Virol* 1985;54:429–435.
44. Sun N, Perlman S. Spread of a neurotropic coronavirus to spinal cord white matter via neurons and astrocytes. *J Virol* 1995;69:633–641.
45. Cserr HF, DePasquale M, Harling-Berg CJ, et al. Afferent and efferent arms of the humoral immune response to CSF-administered albumins in a rat model with normal blood-brain barrier permeability. *J Neuroimmunol* 1992;41:195–202.
46. Hickey WF, Hsu BL, Kimura H. T-lymphocyte entry into the central nervous system. *J Neurosci Res* 1991;28:254–260.
47. Weiss R, Scott F. Pathogenesis of feline infectious peritonitis: Pathologic changes and immunofluorescence. *Am J Vet Res* 1981;42:2036–2048.
48. Pedersen N, Boyle J. Immunologic phenomena in the effusive form of feline infectious peritonitis. *Am J Vet Res* 1980;41:868–876.
49. Jacobse-Geels HE, Daha MR, Horzinek MC. Isolation and characterization of feline C3 and evidence for the immune complex pathogenesis of feline infectious peritonitis. *J Immunol* 1980;125:1606–1610.
50. Weiss R, Scott F. Pathogenesis of feline infectious peritonitis: Nature and development of viremia. *Am J Vet Res* 1981;42:382–390.
51. Vennema H, de Groot RJ, Harbour DA, et al. Early death after feline infectious peritonitis virus challenge due to recombinant vaccinia virus immunization. *J Virol* 1990;64:1407–1409.
52. Hohdatsu T, Nakamura M, Ishizuka Y, et al. A study on the mechanism of antibody-dependent enhancement of feline infectious peritonitis virus infection in feline macrophages by monoclonal antibodies. *Arch Virol* 1991;120:207–217.
53. Olsen CW, Corapi WV, Ngichabe CK, et al. Monoclonal antibodies to the spike protein of feline infectious peritonitis virus mediate antibody-dependent enhancement of infection of feline macrophages. *J Virol* 1992;66:956–965.
54. Corapi WV, Olsen CW, Scott FW. Monoclonal antibody analysis of neutralization and antibody-dependent enhancement of feline infectious peritonitis virus. *J Virol* 1992;66:6695–6705.
55. Haagmans BL, Egberink HF, Horzinek MC. Apoptosis and T-cell depletion during feline infectious peritonitis. *J Virol* 1996;70:8977–8983.
56. Dean G, Woo J, Lavoy A, et al. Cytokine expression in feline lymphoid tissue in health and disease. *Feline immunology workshop*, University of California, Davis, CA, 1997.
57. Goitsuka R, Ohashi T, Ono K, et al. IL-6 activity in feline infectious peritonitis. *J Immunol* 1990;144:2599–2603.
58. Gunn-Moore DA, Caney SM, Gruffydd-Jones TJ, et al. Antibody and cytokine responses in kittens during the development of feline infectious peritonitis (FIP). *Vet Immunol Immunopathol* 1998;65:221–242.
59. Hasegawa T, Hasegawa A. Interleukin 1 alpha mRNA-expressing cells on the local inflammatory response in feline infectious peritonitis. *J Vet Med Sci* 1991;53:995–999.
60. Deli MA, Descamps L, Dehouck MP, et al. Exposure of tumor

necrosis factor- α to luminal membrane of bovine brain capillary endothelial cells cocultured with astrocytes induces a delayed increase of permeability and cytoplasmic stress fiber formation of actin. *J Neurosci Res* 1995;41:717–726.

61. Duchini A, Govindarajan S, Santucci M, et al. Effects of tumor necrosis factor- α and interleukin-6 on fluid-phase permeability and ammonia diffusion in CNS-derived endothelial cells. *J Invest Med* 1996;44:474–482.

62. Stohlman SA, Matsushima GK, Casteel N, et al. In vivo effects of coronavirus-specific T cell clones: DTH inducer cells prevent a lethal infection but do not inhibit virus replication. *J Immunol* 1986;136:3052–3056.

63. Fleming JO, Shubin RA, Sussman MA, et al. Monoclonal antibodies to the matrix (E1) glycoprotein of mouse hepatitis virus protect mice from encephalitis. *Virology* 1989;168:162–167.

64. Wang FI, Stohlman SA, Fleming JO. Demyelination induced by murine hepatitis virus JHM strain (MHV-4) is immunologically mediated. *J Neuroimmunol* 1990;30:31–41.

65. Dörries R, Schwender S, Imrich H, et al. Population dynamics of lymphocyte subsets in the central nervous system of rats with different susceptibility to coronavirus-induced demyelinating encephalitis. *Immunology* 1991;74:539–545.

66. Stohlman SA, Bergmann CC, van der Veen RC, et al. Mouse hepatitis virus-specific cytotoxic T lymphocytes protect from lethal infection without eliminating virus from the central nervous system. *J Virol* 1995;69:684–694.

67. Castro RF, Evans GD, Jaszewski A, et al. Coronavirus-induced demyelination occurs in the presence of virus-specific cytotoxic T cells. *Virology* 1994;200:733–743.

68. Pewe L, Xue S, Perlman S. Infection with cytotoxic T-lymphocyte escape mutants results in increased mortality and growth retardation in mice infected with a neurotropic coronavirus. *J Virol* 1998;72:5912–5918.

69. Shibata S, Kyuwa S, Lee SK, et al. Apoptosis induced in mouse hepatitis virus-infected cells by a virus-specific CD8⁺ cytotoxic T-lymphocyte clone. *J Virol* 1994;68:7540–7545.

70. Schmied M, Breitschopf H, Gold R, et al. Apoptosis of T lymphocytes in experimental autoimmune encephalomyelitis. Evidence for programmed cell death as a mechanism to control inflammation in the brain. *Am J Pathol* 1993;143:446–452.

71. Körner H, Schliephake A, Winter J, et al. Nucleocapsid or spike protein-specific CD4⁺ T lymphocytes protect against coronavirus-induced encephalomyelitis in the absence of CD8⁺ T cells. *J Immunol* 1991;147:2317–2323.

72. Sussman MA, Shubin RA, Kyuwa S, et al. T-cell-mediated clearance of mouse hepatitis virus strain JHM from the central nervous system. *J Virol* 1989;63:3051–3056.

73. Williamson JS, Stohlman SA. Effective clearance of mouse hepatitis virus from the central nervous system requires both CD4⁺ and CD8⁺ T cells. *J Virol* 1990;64:4589–4592.

74. Schwender S, Hein A, Imrich H, et al. On the role of different lymphocyte subpopulations in the course of coronavirus MHV IV (JHM)-induced encephalitis in Lewis rats. *Adv Exp Med Biol* 1993;342:425–430.

75. Lane TE, Liu MT, Chen BP, et al. A central role for CD4(+) T cells and RANTES in virus-induced central nervous system inflammation and demyelination. *J Virol* 2000;74:1415–1424.

76. Perlman S, Sun N, Barnett E. Spread of MHV-JHM from nasal cavity to white matter of spinal cord. In: Talbot P, Levy G, eds. *Coronavirus and Related Viruses*. New York, NY: Plenum Press; 1995:73–78.

77. Sun N, Grzybicki D, Castro RF, et al. Activation of astrocytes in the spinal cord of mice chronically infected with a neurotropic coronavirus. *Virology* 1995;213:482–493.

78. Tracey KJ, Cerami A. Tumor necrosis factor, other cytokines and disease. *Annu Rev Cell Biol* 1993;9:317–343.

79. Lotz M. Interleukin-6: A comprehensive review. *Cancer Treatment Res* 1995;80:209–233.

80. Samoilova EB, Horton JL, Hilliard B, et al. IL-6-deficient mice are resistant to experimental autoimmune encephalomyelitis: Roles of IL-6 in the activation and differentiation of autoreactive T cells. *J Immunol* 1998;161:6480–6486.

81. Akira S, Taga T, Kishimoto T. Interleukin-6 in biology and medicine. *Adv Immunol* 1993;54:1–78.

82. Tilg H, Dinarello CA, Mier JW. IL-6 and APPs: Anti-inflammatory and immunosuppressive mediators. *Immunol Today* 1997;18:428–432.

83. Parra B, Hinton DR, Lin MT, et al. Kinetics of cytokine mRNA expression in the central nervous system following lethal and nonlethal coronavirus-induced acute encephalomyelitis. *Virology* 1997;233:260–270.

84. Lin MT, Hinton DR, Parra B, et al. The role of IL-10 in mouse hepatitis virus-induced demyelinating encephalomyelitis. *Virology* 1998;245:270–280.

85. Parra B, Hinton DR, Marten NW, et al. IFN- γ is required for viral clearance from central nervous system oligodendroglia. *J Immunol* 1999;162:1641–1647.

86. Smith AL, Barthold SW, de Souza MS, et al. The role of gamma interferon in infection of susceptible mice with murine coronavirus, MHV-JHM. *Arch Virol* 1991;121:89–100.

87. Collins AR. Interferon gamma potentiates human coronavirus OC43 infection of neuronal cells by modulation of HLA class I expression. *Immunol Invest* 1995;24:977–986.

88. Lane TE, Asensio VC, Yu N, et al. Dynamic regulation of alpha- and beta-chemokine expression in the central nervous system during mouse hepatitis virus-induced demyelinating disease. *J Immunol* 1998;160:970–978.

89. Sasseville VG, Smith MM, Mackay CR, et al. Chemokine expression in simian immunodeficiency virus-induced AIDS encephalitis. *Am J Pathol* 1996;149:1459–1467.

90. Asensio VC, Campbell IL. Chemokine gene expression in the brains of mice with lymphocytic choriomeningitis. *J Virol* 1997;71:7832–7840.

91. Postorino Reeves N. Vaccination against naturally occurring FIP in a single large cat shelter. *Feline Pract* 1995;23:82–91.

92. Gerber J. Overview of the development of a modified live temperature-sensitive FIP virus vaccine. *Feline Pract* 1995;23:62–66.

93. McArdle F, Tennant B, Bennett M, et al. Independent evaluation of a modified live feline infectious peritonitis virus vaccine under experimental conditions (University of Liverpool experience). *Feline Pract* 1995;23:67–71.

94. Scott F, Corapi W, Olsen C. Independent evaluation of a modified live FIPV vaccine under experimental conditions (Cornell experience). *Feline Pract* 1995;23:74–76.

95. Daniel C, Talbot PJ. Protection from lethal coronavirus infection by affinity-purified spike glycoprotein of murine hepatitis virus, strain A59. *Virology* 1990;174:87–94.

96. Talbot PJ, Dionne G, Lacroix M. Vaccination against lethal coronavirus-induced encephalitis with a synthetic decapeptide homologous to a domain in the predicted peplomer stalk. *J Virol* 1988;62:3032–3036.

97. Koolen MJ, Borst MA, Horzinek MC, et al. Immunogenic peptide comprising a mouse hepatitis virus A59 B-cell epitope and an influenza virus T-cell epitope protects against lethal infection. *J Virol* 1990;64:6270–6273.

98. Koo M, Bendahmane M, Lettieri GA, et al. Protective immunity against murine hepatitis virus (MHV) induced by intranasal or subcutaneous administration of hybrids of tobacco mosaic virus that carries an MHV epitope. *Proceedings of the National Academy of Sciences of the United States of America* 1999;96:7774–7779.

99. Townsend GC, Scheld WM. Adjunctive therapy for bacterial meningitis: Rationale for use, current status, and prospects for the future. *Clin Infect Dis* 1993;17(Suppl 2):S537–S549.