Feline Infectious Peritonitis

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I. INTRODUCTION

Feline infectious peritonitis (FIP) is one of the most intriguing diseases caused by a coronavirus. It involves immune-mediated phenomena such as antibody-dependent enhancement of virus infection- and immune complex-induced pathology. Furthermore, there is increasing evidence for the existence of a carrier state. FIP and feline coronaviruses have been extensively reviewed (Pedersen, 1976b, 1983a,b, 1987a; Barlough and Stoddart, 1986, 1990; Olsen, 1993). Most of these reviews emphasized the pathology, epidemiology, and classical virology. During the last 10 years, our knowledge of the molecular biology of feline coronaviruses has increased considerably. We will summarize clinicopathological findings and the history of FIP research only briefly. The focus will be on the molecular aspects of feline coronaviruses and of FIP pathogenesis.

II. CLINICAL SIGNS AND PATHOLOGY

FIP is a progressive, debilitating lethal disease of domestic and wild Felidae. The disease is characterized by disseminated perivascular pyogranulomatous inflammation and exudative fibrinous serositis in the abdominal and thoracic cavities. The initial signs of naturally occurring FIP are not very characteristic. The affected cats show anorexia, chronic fever, and malaise. Occasionally, ocular and neurological disorders occur. In classical "wet" or effusive FIP these signs are accompanied by a gradual abdominal distension due to the accumulation of a viscous yellow ascitic fluid. The quantity of fluid can vary from a few

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milliliters to well over a liter. There is also the "dry" or noneffusive form of FIP where little or no exudate is present. The wet and dry forms of FIP are different manifestations of the same infection (Montali and Strandberg, 1972). Gross FIP lesions appear as multiple grayish-white nodules (<1 to 10 mm) in the serosal membranes, liver, lungs, spleen, omentum, intestines, and kidneys (Wolfe and Griesemer, 1971; Montali and Strandberg, 1972). Microscopic lesions consist of disseminated foci of necrosis and pyogranulomatous inflammation, frequently located around smaller vessels. These lesions are characterized by accumulations of fibrin and necrotic debris and by perivascular infiltrations of macrophages, neutrophils, and lymphocytes (Wolfe and Griesemer, 1971; Montali and Strandberg, 1972; Hayashi *et al.*, 1977; Weiss *et al.*, 1980).

III. DISCOVERY AND EARLY STUDIES

The early 1960s are usually quoted as the period when FIP was first recognized (Holzworth, 1963; Feldmann and Jortner, 1964). However, the disease was probably seen earlier. In 1912/13, a case of conspicuous abdominal distension due to ascites formation in a domestic cat was reported (Jakob, 1914); the retrospective diagnosis is supported by the description of fever, high specific gravity of the abdominal fluid containing many granulocytes, and ophthalmological signs in this animal (Fig. 1).

The infectious nature of FIP was established by Wolfe and Griesemer (1966). These authors showed that FIP could be produced in specific pathogen-free cats by intraperitoneal inoculation of ascitic fluid collected from diseased cats. Zook *et al.* (1968) and Ward *et al.* (1968) were the first to present evidence supporting a viral etiology. Filtration studies indicated that the causative agent could pass 200-nm pores. In areas of inflammation, viral particles were observed by electron microscopy within or budding into the endoplasmic reticulum of macrophagelike cells (Zook *et al.*, 1968; Ward *et al.*, 1968; Ward, 1970; Pedersen, 1976a). The viral etiology was formally proven by transmission experiments using virus grown in autochthonous peritoneal macrophage cultures (Pedersen, 1976a) and virus suspensions purified by density gradient techniques (Horzinek *et al.*, 1977).

The morphology and morphogenesis of FIP virus (FIPV) was typical of a coronavirus (Zook *et al.*, 1968; Ward, 1970). Serological studies revealed an antigenic relationship between FIPV and established members of the Coronaviridae family, such as porcine transmissible gastroenteritis virus (TGEV), canine coronavirus (CCV), and the human coronavirus (HCV) 229E (Reynolds *et al.*, 1977; Witte *et al.*, 1977; Pedersen *et al.*, 1978; Horzinek *et al.*, 1982). In fact, CCV, TGEV, and FIPV are antigenically so similar that they may be regarded as host range mutants rather than as separate species (Horzinek *et al.*, 1982). Consistent with this view, inoculation of FIPV causes TGE-like lesions in the small intestine of piglets (Woods *et al.*, 1981). Furthermore, FIP-like pyogranulomatous lesions were found in cats experimentally infected with the CCV strain Insvac-1 (McArdle *et al.*, 1992). Another coronavirus of pigs, the porcine epidemic diarrhea virus (PEDV), was found to be antigenically related to FIPV;

FIGURE 1. Probably the earliest case of FIP documented in the literature (at the State Veterinary School in Utrecht, 1912/13); the retrospective diagnosis is plausible from the description of a chronic exudative peritonitis, dyspnea (pleuritis?), fever, and eye symptoms (Jakob, 1914).

serological cross-reactions were confined to the nucleocapsid protein (Zhou *et al.*, 1988).

The isolation of feline coronaviruses (FCoVs) from naturally infected cats is notoriously difficult, probably due to their poor *in vitro* growth; FCoV strains isolated so far are listed in Table I. Since initial attempts to grow the virus in embryonated eggs, primary cells, and continuous cell lines were not successful, FCoV strains were adapted to and propagated in the brains of suckling mice, rats, and hamsters (Osterhaus *et al.*, 1978a,b). *In vitro* propagation was first reported in macrophage cultures (Pedersen, 1976a). A breakthrough was the finding that FCoVs could be grown in continuous lines and primary cultures of feline fetal cells (O'Reilly *et al.*, 1979; Black, 1980; Evermann *et al.*, 1981; Pedersen *et al.*, 1981a). Currently, FCoVs are mostly grown in Crandell feline kidney (CrFK) cells, feline embryonic lung (FEL) cells (O'Reilly *et al.*, 1979), and the line of *Felis catus* whole fetus (fcwf) cells developed by Niels Pedersen; the latter exhibit macrophagelike features at early passage levels (Jacobse Geels and Horzinek, 1983).

TABLE I. Feline Coronavirus Isolates

Strain	Presumptive serotype	Reference
FIPV UCD1 (NW1)	I	Pedersen <i>et al.</i> (1981a)
FIPV UCD2	I	Pedersen and Floyd (1985)
FIPV UCD3	I	Pedersen and Floyd (1985)
FIPV UCD4	I	Pedersen and Floyd (1985)
FIPV UCD5	?	Pedersen (personal communication)
FIPV UCD6	?	Pedersen (personal communication)
FECV UCD	I	Pedersen <i>et al.</i> (1981b)
FIPV TN-406	I	Black (1980)
FIPV Yayoi	I	Hayashi <i>et al.</i> (1981)
FIPV Dahlberg	?	Osterhaus <i>et al.</i> (1978a)
FIPV KU-2	I	Hohdatsu <i>et al.</i> (1991b)
FIPV 79-1146	II	McKeirnan <i>et al.</i> (1981)
FIPV NOR15 (DF2)	II	Everman <i>et al.</i> (1981)
FECV 79-1683	II	McKeirnan <i>et al.</i> (1981)
FIPV Cornell-1	II	Scott (1987)
FIPV KU-1	II	Hohdatsu <i>et al.</i> (1991b)
FIPV Wellcome	?	O'Reilly <i>et al.</i> (1979)

IV. EPIZOOTIOLOGY AND EPIDEMIOLOGY

Although FIP is a disease mainly of domestic cats, it has been reported in several wild felids, such as the lion (Colby and Low, 1970), leopard (Tuch *et al.*, 1974), European wildcats (Watt *et al.*, 1993), caracal, and lynx (Poelma *et al.*, 1971). The cheetah (*Acinonyx jubatus*) is extremely sensitive FIP, a peculiarity attributed to the genetic uniformity in this species (O'Brien *et al.*, 1985).

FIP occurs in domestic cats of both sexes at about the same frequency (Pedersen, 1976c). The disease occurs most commonly in young cats between 6 months and 2 years of age. In cats between 5 and 13 years of age, FIP is less prevalent; but there appears to be an increased incidence in cats older than 14 years (Pedersen, 1976c, 1983a,b; Addie and Jarrett, 1992a). Close contact between cats is required for effective transmission. Virus is shed from the oropharynx and in the feces (Stoddart *et al.*, 1988a,b). Therefore, the infection most likely results from ingestion and/or inhalation of the virus.

Epidemiological studies showed that FCoV have a worldwide distribution (Horzinek and Osterhaus, 1979) and are widespread in the cat population. Antibodies directed against FIPV or a closely related coronavirus are found in 80 to 90% of the cats in catteries and in 10 to 50% of the cats in single cat households (Pedersen, 1976c; Loeffler *et al.*, 1978; Sparkes *et al.*, 1991, 1992; Addie and Jarrett, 1992a,b). The disease itself, however, occurs sporadically: only 5 to 10% of the seropositive cats actually develop FIP (Pedersen, 1976b; Addie and Jarrett, 1992a,b). These findings seem at odds with the fact that certain FIPV strains such as 79-1146 cause FIP in almost 100% of the experimentally infected cats. To explain this discrepancy, it was initially proposed that most cats in the field become infected with an avirulent virus closely related to FIPV. The isolation of two strains, UCD (Pedersen *et al.*, 1981b) and 79-1683

(McKeirnan *et al.*, 1981), that only cause a mild enteric infection but no FIP seemed to support this view. These avirulent viruses were designated feline enteric coronaviruses (FECVs) (Pedersen *et al.*, 1981b, 1984; Pedersen, 1983a). There is now, however, ample evidence that FECV and FIPV are merely virulence variants of the same virus. The FECV isolates are antigenically (Pedersen *et al.*, 1983; Boyle *et al.*, 1984; Fiscus and Teramoto, 1987a; Hohdatsu *et al.*, 1991a,b; W. V. Corapi, personal communication) and genetically (Herrewegh, Horzinek, Rottier, and de Groot, in preparation; see Section VI) indistinguishable from FIPV strains. The antigenic differences between FIPV 79-1146 and FECV 79-1683 (Fiscus *et al.*, 1987; Hohdatsu *et al.*, 1991b) simply reflect FCoV strain variations and do not correlate with their respective pathogenic properties. FECV 79-1683 is antigenically (Pedersen *et al.*, 1984; Hohdatsu *et al.*, 1991a,b; W. V. Corapi, personal communication) and genetically (Herrewegh, Vennema, Horzinek, Rottier, and de Groot, in preparation; see Section VI) more similar to FIPV 79-1146 than to FECV strain UCD. Moreover, attenuated FIPV strains with properties similar to FECV are readily obtained (Pedersen and Black, 1983; Barlough and Stoddart, 1990), and it is now recognized that even FIP-inducing strains vary dramatically in their pathogenicity (Pedersen and Floyd, 1985; Pedersen, 1987b). In the field, highly virulent strains may be as uncommon as completely avirulent strains. It is therefore more appropriate to consider all strains under the general category of FCoV (Barlough and Stoddart, 1990; Addie and Jarrett, 1992b).

The outcome of an FCoV-infection not only depends on the virus strain but also on the infective dose and the route of inoculation (Pedersen *et al.*, 1981a; Pedersen and Floyd, 1985), the age of the host, concurrent viral infections [e.g., with feline leukemia virus (FeLV)] (Hardy and Hurvitz, 1971; Cotter *et al.*, 1975; Pedersen and Floyd, 1985; Pedersen, 1987b), "stress" (Pedersen, 1976b), and, possibly, the genetic predisposition of the host (O'Brien *et al.*, 1985; Addie and Jarrett, 1992a,b). The combined data suggest that FIP is an infrequent manifestation of a common, inapparent infection (Loeffler *et al.*, 1978; Horzinek and Osterhaus, 1979; Pedersen, 1976b; Addie and Jarrett, 1992a,b). From an evolutionary point of view, viruses that cause low morbidity and low mortality have a survival advantage, especially if they can establish persistent infections. There is increasing evidence for the occurrence of asymptomatic carriers of FCoV. Kittens exposed to healthy, FCoV-seropositive cats seroconvert within 2 to 10 weeks (Pedersen *et al.*, 1981b; Addie and Jarrett, 1992a); a number of these kittens subsequently develop FIP. Strong support for the existence of a FCoV carrier state stems from a series of experiments in which FIPV-immune cats were immunosuppressed by superinfection with FeLV. Thus, FIP could be induced in cats that were kept in strict isolation up to 4 months after exposure to FIPV (Pedersen and Floyd, 1985; Pedersen, 1987b). The reverse transcription-polymerase chain reaction (RT-PCR) detection of FCoV in the feces and plasma of healthy, seropositive cats (Herrewegh *et al.*, 1995) provides further support for a FCoV carrier state.

The epidemiological findings fit a model in which most FCoVs, though in principle pathogenic, behave as innocent commensals. The host's immune system (presumably the cellular arm) (Pedersen, 1987b) keeps the infection in

check but is unable to clear the virus completely. Thus, upon natural infection disease would only ensue under exceptional circumstances, i.e., when (1) the host is exposed to a high virus dose, (2) the host is unable to develop a protective immune response, or (3) an immunocompetent host is exposed to factors that compromise its immune system.

V. MOLECULAR BIOLOGY OF FCoV

As for most other coronaviruses, FCoV virions are composed of three main structural protein species: a 45-kDa nucleocapsid protein N, a 25- to 30-kDa matrix protein M, and a 180- to 210-kDa peplomer protein S (Horzinek *et al.*, 1982; Boyle *et al.*, 1984; de Groot *et al.*, 1987a). Virions of TGEV also contain a small membrane protein (sM) of 10 kDa (Godet *et al.*, 1992). Recent data obtained in our laboratory indicate that sM is present in FCoV virions as well (Vennema, Godeke, Horzinek, and Rottier, in preparation). Further details on the characteristics of the structural proteins and coronavirus assembly will be presented elsewhere in this volume (Chapters 5–9).

The FCoV genome is a positive-stranded RNA molecule with an estimated length of about 30 kb. Thus far, the FCoV strain best characterized at the molecular level is FIPV 79-1146. Initially, six virus-specific, poly(A)-containing RNAs were found in infected cells, with lengths ranging from 1.4 to more than 20 kb. An identical set of RNAs was found in cells infected with FIPV strain NOR15 (de Groot *et al.*, 1987a). Recently, we have obtained evidence for a seventh virus-specific RNA species (see below).

By *in vitro* translation, subgenomic RNAs of 3.8 and 2.8 kb were shown to encode the M and N protein, respectively (de Groot *et al.*, 1987a). Sequence analysis of cDNA clones (de Groot *et al.*, 1987b,c) identified the 9.6-kb RNA species to encode the S protein. Of the FIPV 79-1146 genome, the nucleotide sequence downstream of the polymerase gene has now been completed (de Groot *et al.*, 1987b, 1988; Vennema, de Groot, and Spaan, unpublished results). The gene organization is identical to that of CCV strain Insavc-1 (Horsburgh *et al.*, 1992). In addition to the genes encoding N, M, S, and sM, there are five open reading frames (ORFs) (Fig. 2). The sequence 5' AACUAAAC 3' is interspersed between the ORFs; the complementary sequence, 3' GUUUAGUU 5', is thought to function as a promoter for subgenomic RNA synthesis (see Chapter 2).

Interestingly, the sM gene is preceded by the partial promoter sequence 3' GUUUAG 5' (Vennema, de Groot, and Spaan, unpublished results), suggesting that it may be translated from a separate, hitherto undetected RNA species (de Groot *et al.*, 1987a, 1988). Upon separation of FIPV RNAs in 1.5% agarose gels, a minor 4.0-kb RNA species indeed was found. The identity of this RNA was confirmed by RT-PCR using oligonucleotide primers located in ORF4 and the FIPV leader RNA, followed by sequence analysis (Mijnes and de Groot, unpublished results). The mRNA for sM is made in very low amounts, and in 1% agarose gels it is masked by the far more predominant 3.8-kb RNA species. In view of these data it seems appropriate to change the nomenclature of the FIPV

FIGURE 2. Organization and expression of the genome of FIPV strain 79-1146. Open reading frames are indicated by boxes. The structure of the seven FIPV RNAs is also shown.

RNAs as indicated in Fig. 2. The ORFs at the 3' end of the FIPV genome should thus be referred to as 7a and 7b (formerly 6a and 6b), according to nomenclature recommended by the Coronavirus Study Group (Cavanagh *et al.*, 1990).

As illustrated in Fig. 2, RNAs 3 and 7 are potentially polycistronic. ORFs 3a-c and ORFs 7a and 7b are thought to encode nonstructural proteins. It is still unclear, however, whether all of these ORFs, in particular ORFs 3b and c, are actually expressed (Horsburgh *et al.*, 1992). The ORF7a homologue of TGEV contains a 69 nucleotide (nt) deletion compared to ORF7a of FCoV and CCV (de Groot *et al.*, 1988; Vennema *et al.*, 1992b; Horsburgh *et al.*, 1992). Its product, a 9.1-kDa hydrophobic protein, has been detected in TGEV-infected cells (Garwes *et al.*, 1989; Tung *et al.*, 1992). It therefore is likely that ORF7a is expressed in FCoV-infected cells as well.

ORF7b is present only in the genomes of FCoV and CCV (Kapke and Brian, 1986; de Groot *et al.*, 1988; Vennema *et al.*, 1992b; see Chapter 3). It codes for a nonvirion secretory glycoprotein, gp7b (Vennema *et al.*, 1992a). The glycoprotein gp7b is not required for virus replication *in vivo* and *in vitro*: FECV strain 79-1683 contains a large deletion in ORF7b (Vennema *et al.*, 1992b), but replicates to high titers in tissue culture cells and causes enteritis upon experimental infection of cats. Also, ORF7b is readily lost upon *in vitro* passage of other FCoV strains: large deletions in this gene were found in high passage stocks of strains TN406 and UCD4, in UCD2 and in the DF2-derived *Primucell* vaccine strain (Herrewegh, Vennema, Horzinek, Rottier, and de Groot, in preparation). Still, with the exception of strain 79-1683, an intact ORF7b is present, at least at low passage numbers, in all nine FCoV laboratory strains studied thus far, including the nonvirulent FECV strain UCD. Moreover, no deletions were found in ORF7b of 16 field strains as determined by RT-PCR analysis on fecal

samples and tissues (Herrewegh *et al.*, 1995). It therefore appears that although the 7b gene is dispensable for replication in tissue culture cells and enterocytes, its presence provides a selective advantage during natural infection.

The function of gp7b remains enigmatic. In the infected cell, the protein is present only in the lumen of the endoplasmic reticulum (ER) and the Golgi (Vennema *et al.*, 1992a). An involvement in cytoplasmic processes such as RNA replication can therefore be excluded. Presumably, gp7b exerts its function extracellularly, perhaps as a virus-encoded modifier of the immune response and/or inflammatory reaction (Gooding, 1992). The loss of gp7b may well contribute to the reduced virulence of FECV 79-1683, high passage TN406 (Pedersen and Black, 1983), FIPV UCD2, and the *Primucell* vaccine strain.

VI. SEROLOGICAL AND GENETIC RELATIONSHIPS OF FCoV ISOLATES

As first noted by Pedersen *et al.* (1983), FCoVs can be allocated to at least two serotypes on the basis of *in vitro* neutralization. Sera from cats experimentally infected with so-called type I FCoVs (FIPV TN-406 or FECV UCD) neutralized other type I FCoVs but not type II FCoVs (79-1146 and 79-1683) and vice versa. The type II strains appeared to be more closely related to CCV and TGEV, i.e., immunodominant neutralization epitopes shared by the spike proteins of TGEV, CCV, and the type II FCoV strains seemed to be absent in the type I strains (Pedersen *et al.*, 1983). These findings were confirmed and extended in studies using monoclonal antibodies (MAbs) (Hohdatsu *et al.*, 1991a,b, 1992; W. V. Corapi, personal communication). Strains DF-2 (NOR15), CU1 and KU-1 were tentatively identified as type II viruses, whereas UCD-1, -2, -3, and -4, and Yayoi and KU-2 were assigned to serotype I (Table I) (Hohdatsu *et al.*, 1991a,b; W. V. Corapi, personal communication). Strain UCD-2 is interesting in that a number of epitopes on the spike protein, shared by other group I strains, are absent (Fiscus and Teramoto, 1987a,b; W. V. Corapi, personal communication). This led Fiscus and Teramoto to suggest that UCD-2 represents a separate antigenic type (Fiscus and Teramoto, 1987a).

There is some confusion in regard to the classification of FCoV strain UCD1. According to some reports, UCD1 belongs to type I (Pedersen *et al.*, 1983; Hohdatsu *et al.*, 1991a,b). Other studies suggest a closer antigenic relationship to type II strains (Corapi *et al.*, 1992). As it now appears, virus stocks may have been interchanged. The alleged UCD1 strain used by Scotts' group (Corapi *et al.*, 1992; Olsen *et al.*, 1992), is antigenically (W. V. Corapi, personal communication) and genetically more similar to 79-1146 (type II) than to the original type I UCD1 (NW1) strain from Niels Pedersen.

Although the data are limited and rather sketchy, type I FCoV strains seem to predominate in the field. Of 20 sera from natural FIP cases in the United States, only one contained neutralizing antibodies directed against FIPV strain 79-1146 (type II). The remaining 19 antisera neutralized TN-406 (type I) with titers ranging from 40 to 3200 (Pedersen *et al.*, 1983). Hohdatsu *et al.* (1992)

screened 237 FCoV-positive sera, as determined by immunofluorescent assay (IFA), by using a neutralization assay with FIPV strain 79-1146 as the test virus and by a competition enzyme-linked immunosorbent assay (ELISA) using a type II-specific MAb. Their results suggest that type II viruses account for only 20–30% of the natural FCoV infections in Japan.

The type I and II viruses also appear to differ in their *in vitro* growth characteristics. The type I strains grow poorly in tissue culture, yielding titers of cell-free virus of only 0.5 to 1×10^5 TCID₅₀/ml. More than 90% of the infectivity remains cell-associated (Pedersen *et al.*, 1983; Pedersen and Floyd, 1985; Hohdatsu *et al.*, 1991b). In contrast, type II FCoVs grow to high titers of cell-free virus (up to 5×10^7 TCID₅₀/ml) and also in these properties closely resemble CCV (Pedersen *et al.*, 1983).

One interpretation of the data is that the FCoV are a heterogeneous group, with the type II viruses being genetically more closely related to CCV than to type I strains (Pedersen *et al.*, 1983). The fact that some CCV strains induce FIP upon experimental inoculation of cats (McArdle *et al.*, 1992) would lend further credence to this idea. To study this issue, we compared the 3'-most transcription unit comprising ORFs 7a, 7b, and the 3' nontranslated sequences (NTR) of nine different FCoV isolates. Nucleotide sequence similarities ranged from 87 to 99% (Herrewegh, Vennema, Horzinek, Rottier, and de Groot, in preparation). As shown in Fig. 3, types I and II cannot be distinguished in this part of the genome. Clearly, they are much more related to each other than to the CCV strains sequenced thus far.

From serological studies, it appears that the differences between type I and II FCoVs are mostly located in the S-protein (Fiscus and Teramoto, 1987a,b; Hohdatsu *et al.*, 1991a,b; W. V. Corapi, personal communication). Very recently, these findings were confirmed by sequence analysis. The spike genes of type II FCoVs bear much greater resemblance to those of TGEV and CCV than to the spike genes of type I isolates KU-2 (Motokawa *et al.*, 1995) and UCD3 (Vennema *et al.*, 1995). How can these observations be reconciled with those made for the ORF7a/7b transcription unit? During coronavirus replication, homologous RNA recombination occurs at a high frequency. It is quite conceivable that the type II FCoV strains have arisen by RNA recombination between a type I FCoV and CCV (Vennema *et al.*, 1995; Herrewegh *et al.*, in preparation).

Figure 3 also shows that the avirulent "FECV" strains UCD and 79-1683 are more similar to pathogenic "FIPV" strains than to each other. These findings again indicate that the virulent and avirulent FCoVs are not separate virus species.

VII. ANTIBODY-DEPENDENT ENHANCEMENT

The key pathogenic event in FIP is the infection of monocytes and macrophages (Ward, 1970; Pedersen, 1976a). Avirulent FCoV strains remain confined to the digestive tract and usually do not spread beyond the intestinal epithelium and regional lymph nodes (Pedersen *et al.*, 1981b, 1984). The virulent strains,

FIGURE 3. A dendrogram showing the sequence similarities between FCoV and CCV strains. The nucleotide sequence of the 3'-most transcription unit, containing ORFs 7a and 7b, was determined for nine different FCoV strains [Herrewegh *et al.*, in preparation]. The FCoV and CCV sequences were aligned and the tree was constructed using unweighted pair-group method using arithmetic averages [Sneath and Sokal, 1973]. The distance along the horizontal axis is proportional to the differences between sequences.

however, disseminate to other organs most likely via blood-borne monocytes (Weiss and Scott, 1981a). The virulence of FCoV strains appears to correlate with their ability to infect feline peritoneal macrophages *in vitro*. When virulent and avirulent strains were compared, the latter infected fewer macrophages and produced lower virus titers than the virulent strains. Moreover, the avirulent strains were less able to sustain viral replication and to spread to other macrophages (Stoddart and Scott, 1989).

There is ample evidence for an involvement of the immune system in the pathogenesis of FIP. Several authors have emphasized the similarities between FIP and immune-mediated diseases such as Aleutian disease of mink and Dengue hemorrhagic fever (Horzinek and Osterhaus, 1979; Pedersen and Boyle, 1980; Weiss and Scott, 1981b; Halstead, 1988). Humoral immunity is obviously not protective. FCoV-seropositive cats that are experimentally infected with FIPV often develop an accelerated, fulminating course of the disease. Clinical signs and lesions develop earlier, and the mean survival time is dramatically reduced compared to seronegative cats (early death syndrome) (Pedersen and Boyle, 1980; Weiss *et al.*, 1980; Weiss and Scott, 1981a–c). Direct evidence for the involvement of antibodies in early death syndrome was obtained by passive immunization of cats with the purified immunoglobulin IgG fraction of feline α FCoV-antisera. These cats also developed enhanced FIP upon an experimental challenge infection (Pedersen and Boyle, 1980).

Heterologous expression of S, M, and N provided a means to assess the role of each individual protein in FIP pathogenesis (de Groot *et al.*, 1989; Vennema *et al.*, 1990b, 1991). S was unequivocally identified as the main, if not the sole, factor in the induction of early death (Vennema *et al.*, 1990b, 1991). Kittens that had been infected with a recombinant vaccinia virus (recVV) expressing S died within 9 days after an FIPV challenge infection. In contrast, kittens that had been infected with wild-type vaccinia virus or with recVVs expressing M or N either survived FIPV infection or succumbed after a protracted course of the disease (21–41 days) (Vennema *et al.*, 1990b, 1991).

The early death syndrome can be explained by an antibody-dependent enhancement of virus infection (ADE), a phenomenon described for a wide variety of viruses, including the human immunodeficiency virus (Takeda *et al.*, 1988; for reviews, see Porterfield, 1986; Halstead, 1988). Binding of antibody to virus produces infectious immune complexes that attach to the surface of the target cell (via Fc or complement receptors) with higher efficiency than virus alone (Chanas *et al.*, 1982; Burstin *et al.*, 1983; Gollins and Porterfield, 1984, 1985; Halstead and O'Rourke, 1977; Peiris and Porterfield, 1979; Peiris *et al.*, 1981).

Cheryl Stoddart (1989) provided the first *in vitro* evidence for an involvement of ADE in FIP by using feline peritoneal macrophage cultures (Stoddart and Scott, 1988). The number of macrophages infected with FIPV UCD-1* *in vitro* increased up to 12-fold when the virus had been preincubated with serum from an FIPV-sensitized cat. This enhancement also occurred when using the high-pressure liquid chromatography (HPLC)-purified IgG-fraction of the serum. ADE could be prevented by blocking the macrophage Fc receptor with aggregated IgG or by blocking the Fc portion of the enhancing IgG with protein

A (Stoddart, 1989). The levels of enhancement varied greatly between experiments with different batches of macrophages; similar difficulties have been experienced in ADE studies with peripheral blood leukocyte preparations (Porterfield, 1986).

Recently, these observations were confirmed and extended by Hohdatsu *et al.* (1991c) and by Fred Scott's group (Olsen *et al.*, 1992; Corapi *et al.*, 1992). In these studies, ADE in primary feline macrophages was demonstrated both with feline antisera (Olsen *et al.*, 1992; Olsen and Scott, 1993) and with murine monoclonal antibodies (Hohdatsu *et al.*, 1991c; Olsen *et al.*, 1992; Corapi *et al.*, 1992). The variation between experiments was minimized by using batches of macrophages obtained from a single cat (Olsen *et al.*, 1992). Upon screening of 67 MAbs specific for S, M, and N, 17 MAbs were identified that enhanced FIPV infection (Olsen *et al.*, 1992, 1993; Corapi *et al.*, 1992). Consistent with the data of Vennema *et al.* (1990b, 1991), the ADE-inducing MAbs were all directed against the S protein (Olsen *et al.*, 1992; Corapi *et al.*, 1992). There was a distinct correlation between the ability of the MAbs to neutralize infection of CrFK cells and the induction of ADE: 15 of 19 neutralizing antibodies enhanced FIPV infection. All but one of the enhancing MAbs were of the immunoglobulin G2a subclass; the four neutralizing MAbs that did not induce ADE were of the G1 subclass. From these findings Corapi *et al.* (1992) suggested a restriction in the immunoglobulin subclasses mediating ADE. The findings of Hohdatsu *et al.* (1991c) seem to conflict with those of all other authors in that nonneutralizing MAbs directed against both M and S induced ADE. However, the enhancement levels obtained with these MAbs were very low (two- to threefold), while in the studies of Olsen *et al.* (1992) and Corapi *et al.* (1992) some MAbs produced up to a 100-fold increase in the number of infected cells. The most convincing enhancement reported by Hohdatsu *et al.* (1991c) [a sixfold increase in the number of infected cells] notably occurred with a neutralizing S-specific MAb of the immunoglobulin G2a subclass.

There are at least five distinct neutralization sites on the S protein of FIPV, four of which have been implicated in ADE (Corapi *et al.*, 1992). By kinetics-based competitive ELISA, two of the enhancement sites were found to correlate with the previously defined sites A and E/F of the TGEV S protein (Olsen *et al.*, 1992). It was also shown that α TGEV MAbs directed against these sites enhanced FIPV infection *in vitro* (Olsen *et al.*, 1993). By sequence analysis of MAb-resistant mutants and PEPSCAN epitope mapping, amino acid residues involved in the formation of TGEV site A have been localized between amino acid residues 538 and 591 (Correa *et al.*, 1990; Gebauer *et al.*, 1991). The epitopes of the enhancing FIPV site A MAbs are probably located in the homologous region (residues 543–597) (Jacobs *et al.*, 1987) on the FIPV spike (Olsen *et al.*, 1993).

Olsen *et al.* (1992) noted strain variations of ADE in that certain MAbs would enhance FIPV UCD-1* more than 79-1146 and vice versa. This is most likely related to the epitopes recognized by the enhancing MAbs and their relative avidity. These observations may in part explain why FIPV does not always cause antibody-mediated early death in seropositive cats; thus, early death did not occur in kittens that had been inoculated with either FECV UCD, FECV 79-1683, or FIPV UCD-2 prior to a lethal challenge with FIPV 79-1146

(Pedersen *et al.*, 1984; Pedersen and Floyd, 1985; Fiscus *et al.*, 1987). Of the two kittens vaccinated with FIPV UCD-4 and challenged with a lethal dose of FIPV 79-1146, only one developed enhanced FIP (Pedersen and Floyd, 1985; Pedersen, 1987b). Similarly, of four kittens immunized with FIPV UCD-4, only two died of accelerated FIP after a challenge infection with FIPV UCD-1; one kitten died after the normal, protracted course of the disease, and the other one survived. The combined data indicate that a particular virus-antibody combination is required for the development of ADE and early death syndrome.

The mechanism of ADE in FIPV remains to be elucidated. An important question that needs to be addressed is whether the dedicated cell surface receptor of FIPV is involved. Scott and co-workers have argued that the enhanced infection occurs via a very efficient form of Fc γ R-mediated endocytosis that is independent from the FIPV receptor (Corapi *et al.*, 1992; Olsen *et al.*, 1992). This hypothesis is based upon (1) the "sheer magnitude" of the increase in the number of infected cells due to ADE, (2) the observation that the macrophagelike murine IC-21 cells could be infected with FIPV only in the presence of an enhancing MAb (Corapi *et al.*, 1992), and (3) the as yet unconfirmed claim that ADE can be blocked by lysosomotropic amines (Stoddart, 1989). In this model, the nucleocapsid would be released into the cytoplasm by fusion of the viral envelope and the endosomal membrane (illustrated in Fig. 4B).

The above model is difficult to reconcile with other observations made for coronavirus entry. During normal entry, membrane fusion is mediated by the viral spikes; it occurs readily at neutral pH, but is inhibited at lower pH values. Therefore, coronaviruses most likely enter the host cell by fusion at the plasma membrane rather than via the endosomal route (see also Chapter 5). Furthermore, heterologously expressed spike proteins induce membrane fusion in a host cell-dependent manner: the spike protein of FIPV only induced cell fusion in cells of feline origin. Similarly, fusion by the S protein of mouse hepatitis virus was restricted to murine cells (de Groot *et al.*, 1989; Vennema *et al.*, 1990a). These data indicate that membrane fusion is a highly specific process, most likely requiring recognition of the cell surface receptor by the viral spikes (Fig. 4A).

Corapi's experiments with the murine IC-21 cells (Corapi *et al.*, 1992) would suggest that during ADE Fc γ Rs suffice for viral entry. However, it cannot be excluded that IC-21 cells carry a low affinity receptor for FIPV that is only recognized efficiently when virus-antibody complexes are fixed by the Fc γ Rs at the plasma membrane. Support for the existence of FIPV receptors on murine cells stems from studies in which FIPV strains were adapted to murine Sac⁻ cells (de Groot, unpublished results) or propagated in brains of suckling mice (Osterhaus *et al.*, 1978a).

We currently favor a model for ADE in which viral entry is mediated by interaction between the spike and the virus receptor, where the probability of this event is increased due to a more efficient binding of the opsonized virus to the cell surface (explained in Fig. 4C). This mechanism would be similar to that found for human immunodeficiency virus (HIV): ADE of HIV infection requires both the Fc γ R and the HIV cell receptor, CD4 (Takeda *et al.*, 1990; Perno *et al.*, 1990; Zeira *et al.*, 1990; Connor *et al.*, 1991).

FIGURE 4. Models for viral entry during (A) normal infection and (B,C) ADE. (B) Model for ADE, in which entry occurs via the endosomal route. Adsorption and penetration requires only the Fc receptor. (C) Model for ADE, in which viral entry requires both the Fc receptor and the virus receptor.

Clearly, the receptor for FIPV must be identified before this issue can be resolved. Two closely related viruses, TGEV and HCV 229E, adsorb to and enter their host cell via the aminopeptidase N (APN) molecule, a membrane-bound metalloprotease (Delmas *et al.*, 1992; Yeager *et al.*, 1992). The tissue distribution of APN coincides with the cell tropism of FIPV; it is present on enterocytes and macrophages (Look *et al.*, 1989). It is thus quite likely that FIPV utilizes APN as a receptor as well.

VIII. IMMUNE-MEDIATED LESIONS IN FIP

Most authors consider the vascular and perivascular lesions in FIP to be immune-mediated, but there is uncertainty about the actual pathogenic mechanism. At least some vascular injury may be attributed to immune-mediated lysis of infected cells (Weiss and Scott, 1981a): FIPV-infected white blood cells were detected in the lumen, intima and wall of veins and in perivascular locations (Weiss and Scott, 1981a; Weiss *et al.*, 1988). Furthermore, inflammatory mediators such as cytokines (Goitsuka *et al.*, 1988, 1990; Hasegawa and Hasegawa, 1991), leukotrienes, and prostaglandins (Weiss *et al.*, 1988) that are released by infected macrophages could play a pivotal role in the development of the perivascular pyogranulomata. As hypothesized by Weiss *et al.* (1988), these products could induce vascular permeability changes and provide additional chemotactic stimuli for neutrophils and monocytes. In response to the inflammation, the attracted cells may release additional mediators and cytotoxic substances; the monocytes would also serve as new targets for FIPV. The end result of this would be enhanced local virus production and increased tissue damage.

Other observations point toward an immune complex pathogenesis. Deposition of immune complexes and subsequent complement activation is thought to cause an intense inflammatory response that may extend across blood vessel walls. The resulting vascular damage would permit leakage of fluid into the intercellular space and eventually lead to the accumulation of thoracic and abdominal exudate (Hayashi *et al.*, 1977; Horzinek and Osterhaus, 1979; Jacobse-Geels *et al.*, 1980, 1982; Pedersen and Boyle, 1980; Weiss *et al.*, 1980; Weiss and Scott, 1981a–c). The morphological features of the vascular lesions (necrosis, polymorphonuclear cell infiltration associated with small veins and venules) strongly indicate an Arthus-type reaction (Hayashi *et al.*, 1977; Weiss and Scott, 1981c). The lesions contain focal deposits of virus, IgG, and C3 (Pedersen and Boyle, 1980; Weiss and Scott, 1981b). Moreover, complement depletion and circulating immune complexes were demonstrated in cats with terminal FIP (Pedersen and Boyle, 1980). In a horizontal study of six experimentally infected cats, first clinical signs were accompanied by increased C3 concentrations in the plasma; subsequently, antibody titers and circulating immune complexes (ICX) increased with concomitant decrease of complement concentrations. At the time of death, maximum ICX and minimum C3 concentrations were measured (Jacobse-Geels *et al.*, 1982). Glomerular ICX deposits were found in FIPV-infected cats but not in uninfected controls (Jacobse-Geels *et al.*, 1980).

IX. VACCINE DEVELOPMENT

The development of vaccines against FIPV is cumbersome and has been frustrated by the occurrence of ADE. Vaccination with heterologous live virus vaccines (TGEV, CCV, HCV 229E) has failed to provide protection (Witte *et al.*, 1977; Toma *et al.*, 1979; Woods and Pedersen, 1979; Barlough *et al.*, 1984, 1985).

Occasionally, protective immunity can be induced by administering FECV, low virulent FIPV (e.g., FIPV UCD-3), or sublethal amounts of virulent FIPV (Pedersen and Black, 1983; Pedersen *et al.*, 1984; Pedersen and Floyd, 1985; Pedersen, 1987b). However, as quoted from Pedersen and Black (1983, p. 20), the results of immunization with homologous live virus vaccines were "too inconsistent and hazardous to have clinical relevance": while some cats became solidly immune, others developed disease or became sensitized. Also, cats immunized with recombinant vaccinia viruses expressing the spike gene of FIPV 79-1146 (Vennema *et al.*, 1990b) or of FECV 79-1683 (Bhogal, Martinez, Reed, Klepfer, Jones, Pfeiffer, and Miller, in preparation) developed early death syndrome upon an FIPV challenge infection.

A temperature-sensitive strain of FIPV has been shown to stimulate protective immunity in cats. Upon intranasal inoculation into cats, virus replication appeared to be restricted to the upper respiratory tract. The mutant did not differ from the wild-type virus with respect to RNA and protein synthesis, but produced little progeny at the nonpermissive temperature (Christianson *et al.*, 1989; Gerber *et al.*, 1990). There is still controversy over the safety and efficaciousness of this vaccine in that protection may depend on the strain and dose of the challenge virus (Olsen, 1993). Furthermore, even as avirulent and immunizing modified live FIPV strains become available, their widespread use as vaccines is not encouraged in view of the notorious genetic flexibility of coronaviruses. Thus it has been demonstrated that new epidemic strains of avian infectious bronchitis virus have arisen by point mutations in the genomes of attenuated vaccine strains (Kusters *et al.*, 1987). While this may be acceptable in production animals where new vaccine strains can be employed at intervals, the high individual value of a companion animal would plead against the use of live coronavirus vaccines. The possible risks are best illustrated by outbreaks of an unprecedented FIP-like disease in dogs after the widespread use of a modified live CCV vaccine (Martin, 1985).

Designing safe and effective FIP vaccines that avoid immunopathology will probably require a better understanding of the complex interplay between the virus and the host immune system and, perhaps just as important, identification of the viral proteins involved. The molecular cloning of the FIPV genome and the isolation of viral genes provides powerful tools to tackle these problems. Obviously, the S protein is not the most attractive candidate for vaccine development (Vennema *et al.*, 1990b; Bhogal, Martinez, Reed, Klepfer, Jones, Pfeiffer, and Miller, in preparation). The role of M in immunity against FIPV is still unclear. Upon immunization of eight cats with a vaccinia virus recombinant expressing M, three kittens survived, while two other kittens showed protracted survival times compared to the controls (Vennema *et al.*, 1991). Clearly, these findings deserve further scrutiny.

X. CONCLUDING REMARKS

Substantial progress has been made in FIP research. Our knowledge of the molecular genetics of FCoV has increased considerably as has our understand-

ing of the mechanism of ADE and early death syndrome. Other areas would require closer examination, for instance, the role of cellular immunity in protection against FIP. As feline cytokine genes are cloned and expressed, new tools become available to modulate the feline immune response and possibly also FIP pathogenesis. Another interesting field is the presumptive FCoV carrier state. It remains to be determined whether FCoVs can cause a true persistent infection, and if so, in which cells and tissues of the host the virus resides and how it manages to escape immunosurveillance. Finally, FCoVs produce a number of proteins that are dispensable for replication in tissue culture cells, most notably gp7b. What is the function of these proteins during natural infection? Though the last ten years of FIP research have been exciting, the coming years promise to be better still.

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