CHAPTER 10

Feline Coronavirus Infections

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ETIOLOGY

Feline coronavirus (FCoV) causes a ubiquitous enteric infection of cats that occasionally leads to a highly fatal immune-mediated vasculitis named feline infectious peritonitis (FIP). FCoV is a large, spherical, enveloped, positive-sense single-stranded RNA alphacoronavirus (previously termed "group 1") belonging to the family Coronaviridae. The Coronaviridae, comprising the genera *Coronavirus* and *Torovirus*, is part of the Nidovirales order, which also includes toroviruses, arteriviruses, and roniviruses.[60] It is proposed that FCoV, along with the coronaviruses of swine and dogs, become part of a new species, called *Geselavirus*, in reference to the typical genetic arrangement of these viruses "*g*ene *se*ven *la*st" (gsl).[60] Coronaviruses possess the largest RNA viral genome known to date: the FCoV genome is 29 kb, encoding a replicase polyprotein, four structural proteins (spike [S], matrix [M], nucleocapsid [N], and envelope [E]), and several nonstructural proteins (3a, 3b, 3c, 7a, and 7b), whose function is unknown.

Despite the ubiquitous nature of FCoVs and infected cats, few develop FIP (Fig. 10-1). The explanations proposed for this discrepancy have been controversial and revolved around two basic premises: whether both avirulent and virulent viruses are simultaneously circulating, or virulent viruses arise as a result of de novo mutation within each FIP-affected cat. In this latter theory, a novel mutation, deletion, or insertion must occur in the genome of the infecting FCoV or feline enteric coronavirus (FECV) before FIP can occur.[45,244,254] Chang et al. found deletions in the 3c gene from systemic virus, but not from virus in the gut, postulating that an intact 3c gene is essential for viral replication in the gut.[45] Pedersen also found intact 3c genes in isolates from the gut.[244] In contrast, others having found both deletions/mutations and identical genomes in healthy and FIP cats, or from both systemic and enteric viruses, have questioned the internal mutation theory.[38,73,186]

Although sharing only 30% genetic homology,[254] the 3c gene has been likened to the 3a gene of severe acute respiratory syndrome-related (SARSr)-coronavirus (CoV) on the basis of hydrophilic profile similarity.[45,223] The SARSr-CoV 3a protein has been implicated in apoptosis; type 1 interferon (IFN) receptor downregulation, and increased fibrinogen expression.[190,206,316] Whether or not deletions in the 3c gene are responsible for the development of FIP is unknown—they could simply be a by-product of rapid viral replication, and 3c deletion mutants made successful vaccine candidates.[107]

RNA viruses are remarkably prone to genetic change, and it would be expected that in a situation where there is considerable viral replication, many variants would be found in the same host. Such variation is found[103,168] not only within organs in the body, but also within different cells in the same pyogranuloma.[262] Whether that virus variation is the cause of, or the effect of, the disease process is unknown. Laboratory strains of varying virulence exist; there are strains that are exceptionally virulent, causing FIP in almost every cat infected with them (e.g., the notorious 79-1146 strain). Less virulent FCoV strains vary in their ability to replicate in monocytes, *and* monocytes vary in their permissiveness for FCoV replication. The interplay of these two factors determines whether or not an individual cat develops FIP.[64] Consistently, cats challenged with low viral dose, even with virulent virus such as FIP virus (FIPV) 79-1146, can overcome the infection, whereas increasing doses resulted in almost all cats developing FIP.[247,286]

Results from a comprehensive genetic analysis of FCoV strains indicated distinct genetic differences between viruses isolated from 48 clinically healthy cats and 8 ill cats with FIP.[38] These distinct noncontiguous differences existed in membrane, spike, and nonstructural protein 7b genes. Unfortunately, the 3c genes were not examined. The membrane protein is the most abundant structural protein of the coronaviruses and is likely associated with the pathogenesis of infection, because it is involved in viral budding. Significantly, there were five amino acid differences between the membrane proteins of FCoVs from clinically healthy cats and those with FIP. However, three healthy cats had viruses that contained an FIP amino acid signature (YIVAL), raising the possibility that at least one cat eliminated a FCoV strain capable of causing FIP.[38] The genotypes correlated with FIP were more compatible with ancestrally derived and not the result of de novo mutations.[38] The majority of cats were not co-infected with multiple strains of FCoVs at the same time, but were generally infected with one predominant strain. However, in two instances cats with FIP were infected with two distinct viral isolates, indicating that superinfection can occur.

Another member of the Coronaviridae causes severe acute respiratory syndrome (SARS) in humans. The SARSr-CoV is thought to have originated from the masked palm civet cat (*Paguma larvata*). Despite its common name, this carnivore is not a feline, but is a member of the mongoose family (Viverridae). Nevertheless, cats may become infected with SARSr-CoV experimentally[196] and naturally. A single cat from one household of infected people was found to seroconvert, although it remained clinically healthy. Analysis of data suggest that civet SARSr-CoV is likely a recombinant virus arising from SARSr-CoV strains closely related to the coronaviruses of the horseshoe bat, *Rhinolophus sinicus*. Frequent recombination coupled with rapid evolution in these animals may have accounted for the cross-species transmission and emergence of SARS.[181]

Feline Coronavirus Serotypes I and II

There are two types of FCoVs, as classified by their genetic sequence and ability of monoclonal antibodies to recognize them.[127,248,316a] Type I FCoVs are considered to be unique feline strains. Type II FCoVs

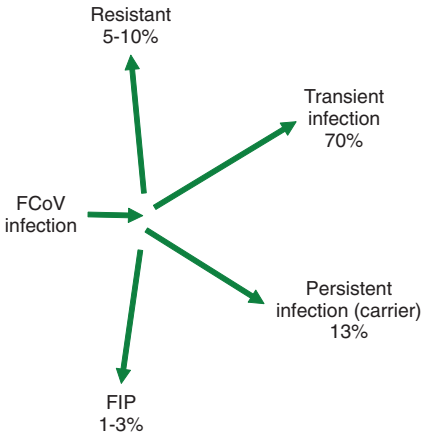

FIG. 10-1 There are four possible outcomes to FCoV infection, and only in a minority of infections is FIP the outcome. The percentage of cats that will have each outcome is shown. (Modified from Addie DD, Jarrett O. 2001. *Vet Rec* 148:649–653.)

have arisen from recombination between type I FCoV and canine coronavirus (CCoV). Although type II FCoVs are mainly type I, they have variable portions of the spike and adjacent genes of CCoV.[118,317] Most research has focused on type II because it can be readily propagated in vitro; however, type I is most prevalent worldwide.* Both types can cause FIP. Some investigators[126] found a higher prevalence of type II among cats with FIP than among healthy cats in Japan, and others[187] found a higher correlation of type II with FIP. However, in other studies the distribution of types I and II in cats with FIP reflected broadly the distribution of the two viral types in asymptomatic FCoV infected cats.[31,178] Lin et al. also found a higher genetic diversity among type I FCoVs compared with type II—a feature they attributed to type I FCoV being able to induce persistent infection, whereas type II FCoV probably does not.[187] Cats can be simultaneously infected with both types I and II FCoV.[187]

The type I FCoV receptor is unknown.[73,123] The receptor for the type II FCoV is an enzyme, aminopeptidase-N, found in the intestinal brush border.† However, at least in type II FCoV infection of monocytes and macrophages, the receptor is not necessary if there is anti-FCoV antibody present.[313]

EPIDEMIOLOGY

FIPV rivals feline panleukopenia virus as a cause of cat death.[42] The apparent increase in the prevalence of FIP can be directly related to the changes in feline husbandry over the past 30 years—more cats are kept indoors and in greater numbers, causing exposure to higher doses of pathogens in feces, which would otherwise have been buried outdoors. The popularity and resulting increased breeding of purebred cats has resulted in loss of immune protection associated with genetic diversity and hybrid vigor.[188] An increasing number of cats spend part of their lives in shelters. This life style can result in exposure to a higher coronaviral dose (via the litter tray), increased stress to the naturally solitary cat, and concurrent, sometimes immunosuppressive infections, all of which impair a cat's ability to prevent infections. Cats are increasingly being prevented from hunting and are instead being fed unnatural foods, often imbalanced in the ratio of omega 6:3 dietary fatty acids, which likely leads to a chronic proinflammatory state. All of these factors favor the spread and increase of FCoV infections and associated FIP. The predominant risk factors associated with the development of FIP are discussed next.

Age

Although a cat of any age can develop FIP, kittens and cats up to 2 years of age are at greatest risk,[215,244,274] with a second peak in age-related risk in cats over 10 years of age.[274] More specifically, kittens developed FIP after weaning,[42] and most young cats succumb between 3 and 16 months of age.[244] That kittens are at greater risk of developing FIP may be due to the higher viral load generally found in kittens compared with adult cats[246]; due to their immature immune systems; or due to the many stressful events that generally happen to kittens, such as being vaccinated, rehomed, and neutered. In addition, cats are most likely to develop FIP after their first encounter with FCoV, which is most likely to occur in kittenhood.[18]

Breed

There is little doubt that pedigree cats are more at risk of developing FIP than are nonpurebred cats.* This may be because purebreeding of cats is associated with the loss of genetic diversity[188] so that the immune systems of purebred cats may not be as robust as those of outbred cats, indeed a study of the feline leukocyte antigen (FLA, the feline equivalent of the major histocompatibility complex [MHC]) showed that the Burmese breed averaged 2.8 FLA alleles, compared with up to 6 in other breeds.[11] Or it may be because cat breeders usually have several cats and they tend to be confined indoors—increasing viral dose to which they are exposed, concurrent stress, and diseases. Cat breeders in the United States[344] and 8% of Swedish cat breeders report having had a cat with FIP.[304]

Results differ between studies—in one study the inheritance of FIP susceptibility was demonstrated in Persian cats.[83] In another study Persian, Burmese, exotic shorthair, Manxe, Russian blue, and Siamese cats were not at increased risk of developing FIP, whereas Abyssinian, Bengal, Birman, Himalayan, ragdoll, and rex cats were.[259] In a retrospective study of neurologic disorders, Burmese cats were overrepresented as having FIP.[36] However, this study was conducted in association with a Burmese cat club, which may have skewed the results for this breed.

Nondomestic Felidae

FCoV infection, disease, and FIP have been reported in a variety of nondomestic felids: European wildcats (*Felis silvestris*),[337] lions (*Panthera leo*),[122,151] tigers (*P. tigris*), jaguars (*P. onca*), leopards (*P. pardus*), sand cats (*Felis margarita*), mountain lions/panthers (*F. concolor*),[234,273] caracals (*Caracal caracal*), and servals (*Felis serval*)[143]; lynx (*Lynx lynx*) in Canada,[33] but not Eurasian lynx in Sweden[282]; one bobcat (*Lynx rufus*)[270]; and especially cheetahs (*Acinonyx jubatus*).† As with domestic cats, FCoV is more likely to be a problem in large cats confined indoors or in exhibits than in those allowed to roam outside naturally.‡

Other Pet Species Coronaviruses

Ferrets have two manifestations of coronavirus infection: epizootic catarrhal enteritis[342,343] and infectious peritonitis.[88,144b,197,258] Although in the same group (1) as FCoV, ferret coronavirus is distinct from FCoV,[343] so one would not expect cross-infection between ferrets and cats in the same household; however, RNA viruses are prone to recombination, so it can possibly occur.[1]

*References 15, 31, 126, 178, 187, 268, 289.
†References 29, 113, 174, 312, 319, 320.

*References 83, 259, 274, 287b, 287c, 297, 344.
†References 115, 155, 150, 151, 154, 217, 260.
‡References 78, 111, 143, 151, 217, 260.

Dogs frequently serve as a source of infection of CCoV. Coronaviruses are frequently transmitted between dogs and cats living in close contact,[30,268] giving rise to recombinant variant viruses.[118,334] (See Feline Coronavirus Serotypes I and II, discussed earlier.) For more information on CCoV, see Chapter 8.

PATHOGENESIS

Virus Shedding

Virus is shed in the feces from 2 days postinfection.[246] It is thought that primary viral replication occurs in the epithelial cells of the small intestine,[243] but in long-term viral excretion, virus is localized in the ileocecocolic junction.[117] A small number of cats are resistant to FCoV infection.[10,64,246] It is likely that viral shedding of types I and II is different; laboratory strains, which are typically type II, are shed for only a couple of weeks,[303] whereas in natural infection, type I virus is shed by 65% of cats for 2 to 3 months or longer by many cats.[10,15] Some cats are co-infected with both types I and II.[187] The majority of cats clear the virus after 2 to 3 months of fecal shedding, although in some infected cats (13%) the virus establishes a persistent infection.[10,15,246] Experimental infection of specific-pathogen free cats with nonvirulent FCoV resulted in persistent localization of the virus in the colonic epithelium, and to a lesser degree in macrophages of the liver and mesenteric nodes, associated with prolonged fecal shedding.[166a] A curious feature of lifelong carrier cats is that they shed the same strain of virus continuously in the feces until death[15]; this is very similar to the situation with chronic carriers of feline calicivirus.[52] FCoV carriers rarely develop FIP.[15] Chronic carrier cats usually appear to remain in adequate health, though some develop chronic large intestinal diarrhea and fecal incontinence in older age.[13] Detection of carrier cats requires positive fecal reverse transcriptase (RT)-polymerase chain reaction (PCR) test results for 9 months.[10]

Virus is maintained in the cat population by chronic carrier cats and through reinfection of transiently infected cats.[10,15,85] The stress of entering a rescue shelter increases viral shedding 10^1- to 10^6-fold.[256] However, the stress of pregnancy and lactation did not cause infected queens to shed more virus.[84] In healthy cats, virus is only shed in the saliva for a very brief period of time (hours).[10] Not all (up to 75%) cats with FIP shed virus in the feces,[17,45,254] and possibly also in other excretions, such as urine, saliva, and tears. Virus shed in the feces tends to have an intact 3c gene.[45,254]

Although serologic (antibody to FCoV) testing has limitations (see later discussion), it is clear that cats with seronegative results, as determined by a *reliable* diagnostic test, do not shed FCoV,[7,10,84] whereas approximately one in three cats with seropositive results does shed virus.[7] Cats with higher antibody titers are more likely to shed virus,[10,84,246] although cats with relatively low indirect fluorescent antibody (FA) titers of 40 to 80 have a 26% to 39% chance of shedding FCoV.[11,12,16]

Evidence of viral shedding is never a good reason to euthanize a cat because most FCoV shedders stop within a few months, and fewer than 10% develop FIP.[8] In addition, if a cat has survived one exposure to FCoV, it may be better to use that animal for breeding rather than introduce new susceptible animals that may not be resistant, because a genetic element may play a role in susceptibility to FCoV infection.[244]

Transmission

Cats become infected with FCoV orally, usually indirectly by contact with cat litter contaminated with the virus. FCoV is a highly infectious virus, and in a multicat household, over 90% of cats will seroconvert. FCoV can survive for 7 weeks in a dry environment.[139] FCoV is readily inactivated by most household detergents and disinfectants; however, bleach is preferred not only because it is efficacious, but also because it is safe for use around cats.[13,139]

FCoV has been isolated from a 1-day-old kitten, implying that transplacental transmission could be possible. However, the practice of removing kittens from infected queens, even those who died of FIP, protected the kittens from infection, which would not have worked had transplacental transmission occurred.[7,139]

Monocyte Infection and Vasculitis

Initially, the development of FIP was attributed to properties of the virus, rather than of the host: less virulent laboratory strains have less ability to replicate in monocytes compared with more virulent strains.[64] However, monocytes of different cats will support FCoV replication to varying extents[64] and some cats' monocytes will not support viral replication at all, which could be an explanation for the occurrence of FCoV-resistant cats as previously reported.[10] Another explanation could be that some cats lack the as yet undetermined receptor for the type I virus. Discoveries regarding the pathogenesis of FIP have been useful in understanding how clinical signs develop and for devising new strategies for therapy.

Using immunohistochemistry, Kipar et al.[166] demonstrated FCoV within monocytes adhering to blood vessel walls and extravasating (Web Fig. 10-1)—this is the key event in the development of FIP. FCoV-infected macrophages release interleukin (IL)-6,[97] IL-1β matrix metalloproteinase (MMP)-9,[166] and tumor necrosis factor (TNF)-α.[166,311,312] In early infection, IL-6 stimulates hepatocytes to release acute-phase proteins (such as alpha-1 acid glycoprotein [AGP]) and B lymphocytes to proliferate and differentiate into plasma cells.[311] It is likely that high IL-6 levels found in cats with FIP are the cause of hypergammaglobulinemia.

TNF-α is a major contributor to the inflammatory response and pathogenesis of FIP. TNF-α is very likely the cause of the lymphopenia seen in FIP,[311] especially in noneffusive FIP. In vitro, apoptosis of lymphocytes (especially CD8+ lymphocytes) that was induced by ascitic fluid, plasma, and culture supernatant of peritoneal exudate cells from cats with FIP was attributed to TNF-α.[311] However, in another study, use of anti-TNF-α or TNF-α neutralizing antibodies was unable to block FIP-induced lymphocyte apoptosis.[105] TNF-α upregulates fAPN (the receptor for type II FCoVs)[312] and, along with granulocyte-macrophage colony-stimulating factor and granulocyte colony-stimulating factor, which are also produced by FCoV-infected monocytes, is a neutrophil survival factor.[312] In later infection, TNF production shifts from macrophages to lymphocytes.[55] Chronic overproduction of TNF-α also results in cachexia.

IL-1 activates B and T cells, is pyrogenic, and contributes to the inflammatory response. MMPs are zinc-dependent endopeptidases capable of breaking down extracellular matrix proteins. It is probable that MMP-9 is responsible for the leakiness of the blood vessels in effusive FIP.

Immune Response to Feline Coronavirus Infection

In addition to the virulence of the infecting strain of FCoV, reduced immunity can predispose a cat to develop systemic infection. Most cats that develop FIP have a history of stress in the previous few months. Stress likely has two effects that increase the cat's susceptibility to FIP: it decreases the immune system, and increases viral shedding 10^1- to 10^6-fold.[256] Furthermore, it is hypothesized that the type and strength of immune response determine the outcome of FCoV infection: that a strong cell-mediated immune (CMI) response will prevent FIP, a weak or nonexistent CMI and strong humoral response results in effusive FIP, and an intermediate response results in noneffusive FIP.[244] Lesions of noneffusive FIP predominate within the eye

and central nervous system (CNS), both sites protected from the immune system.

Evidence from experimental infections showed that cats surviving a challenge mount a greater CMI response than those who succumb.[62] However, clearance of natural infections also correlated with the presence of a humoral immune response to the FCoV spike protein,[103] and it is known that kittens are protected by maternally derived antibody (MDA).[7] Therefore, it is possible that some antibody protection also occurs. Humoral immunity associated with secretory IgA is suspected to be important in preventing initial infection of epithelial cells. However, in exposed cats, seroconversion occurs within 18 to 21 days postinoculation,[204] which is long compared to most viral infections where antibodies appear 7 to 10 days postinoculation. Although some viruses continually mutate as a means of evading the host immune response, cats persistently infected with FCoV shed the same strain for years.[15] Therefore, FCoVs have developed means to suppress the host immune response. It is also evolutionarily beneficial to the virus to delay the humoral response in some way, so that cats become persistently infected and shed virus for longer. Because cats with FIP die and so no longer shed virus, which is not in the evolutionary interest of the virus, FIP might actually be considered as an "evolutionary accident." Further evidence for viral-associated immunosuppression and impaired clearing of virus is that FCoV-infected cats that succumb to FIP have much higher systemic viral levels than those that survive the infection.[159]

The means by which FCoVs suppress the host immune response have not been completely elucidated. As stated previously, one way FCoV affects the host's response is that FCoV-infected cells release a substance that causes apoptosis of lymphocytes,[105] and this substance is likely to be TNF-α.[311] Once antibodies are present, they cause viral proteins on the surface of the monocyte to be internalized within minutes.[50] Perhaps the reason for this is to delay as long as possible the development of anti-S antibodies that are capable of clearing infection.[100]

Antibody-Dependent Enhancement

Antibody-dependent enhancement (ADE) is a phenomenon that has foiled many attempts to find a successful FIP vaccine and is worrisome to those trying to develop a SARS vaccine.[272] In ADE, a greater proportion of cats that had been vaccinated with trial vaccines developed FIP than cats in the unvaccinated control group also exposed to a laboratory strain of FCoV, usually the very virulent 79-1146 type II strain. This strain is not useful because of its extreme virulence. The reason for ADE is not well understood, but one hypothesis is that it is mediated by subneutralizing antibodies that facilitate viral entry into their target cell, the macrophage, via an Fc-receptor-mediated mechanism.* Research shows that addition of antibody to infected macrophages causes rapid internalization of viral proteins from the cell surface.[50,65,66,325,326] The significance of this has not yet been fully elucidated, because it is not to evade antibody dependent complement-mediated lysis of infected cells.[51]

Cats with ADE develop disease in fewer than 12 days, whereas controls take 28 days or more.[285] By contrast, field studies have shown that seropositive pet cats that were naturally reinfected by FCoV showed no evidence of ADE.[15,18] Indeed, many cats that had become seropositive after natural infection appeared to be resistant to developing FIP (though not to reinfection by the same or another strain of FCoV).[15,18] The mortality rate of cats that were in contact at the time of initial FCoV infection was 14%, compared with about 8% at the time of reinfection.[18] In practical terms, a seronegative cat introduced into a household in which FCoV is endemic has a 1 in 6 chance of developing FIP, whereas a seropositive cat has a 1 in 12 chance. Cats are at greatest risk of developing FIP in the first 6 to 18 months after infection, and the risk decreases to about 4% by 36 months after infection.[18] There is no evidence that the available vaccine against FIP (Primucell, Pfizer) causes ADE (see later discussion). Because ADE has been reported experimentally in cats passively given anti-FCoV antibodies,[314] it would be prudent to ensure that blood donors have FCoV seronegative results.

*References 48, 49, 125, 219, 221, 222, 314.

CLINICAL FINDINGS

Initial Infection

Most FCoV infections are subclinical. When FCoV first infects cats, they may have a brief episode of upper respiratory tract signs or diarrhea; although these signs are usually not severe enough to warrant veterinary attention, the diarrhea can occasionally be extremely severe.[164] Kittens infected with FCoV generally have a history of diarrhea and occasionally of stunted growth and upper respiratory tract signs.[7]

Coronavirus Enteritis

Experimentally infected specific-pathogen free cats had diarrhea due to FCoV and can manifest during primary infection, in persistently infected (carrier) cats, and where noneffusive FIP has caused lesions within the colon. Diarrhea, and occasionally vomiting, occurs in kittens and some cats at primary FCoV infection, is a small intestinal diarrhea, and is usually self-limiting within a few weeks. However, occasionally the virus can be responsible for a severe acute or chronic course of vomiting or diarrhea with weight loss, which may be unresponsive to treatment, continue for months, and occasionally result in death.[164] However, there are many other causes of diarrhea in cats that should be considered before a diagnosis of FCoV diarrhea can be made (e.g., *Tritrichomonas foetus*, which tends to affect the same group of cats—young cats living in crowded multicat environments).[101] FCoV diarrhea most frequently presents in young kittens from 5 weeks of age.

Chronic, large-intestinal diarrhea has been noted in older, otherwise healthy, FCoV carrier cats; it may result in fecal incontinence.[3] For details of diarrhea due to FIP, see the later section on Colonic or Intestinal Localization.

Multisystemic Inflammatory Vasculitis Disease

FIP is a misnomer, because many cats do not have peritonitis. Two basic forms of FIP, effusive (wet) and noneffusive (dry), have been characterized. It would be more accurate, however, to think of FIP as a continuum, because they are gradations of the same process, which is basically a pyogranulomatous vasculitis. The clinical and pathologic signs that occur in FIP are direct consequences of the vasculitis and organ damage that result from damage to the blood vessels that supply them. In effusive FIP, many blood vessels are affected, hence the exudation of fluid and plasma proteins into the body cavities. In noneffusive FIP, the clinical presentation depends on which organs are damaged by the FIP pyogranulomata.

Web Fig. 10-2 details the FIP-diagnosis algorithm. In step 1 of the algorithm, cats with FIP tend to be young, from multicat environments (breeding and boarding catteries, rescue shelters, veterinary clinics), and have a history of recent stress; FIP incubation is from weeks to months. Approximately one-half of the cats with FIP are younger than 2 years, but cats of any age can be affected.[215,244,274] Evaluation of the history of cats with FIP typically reveals that they lived in a multicat environment within the previous year, usually with a cat breeder or in a rescue shelter. Occasionally, they have been to a boarding cattery, cat show, or veterinary clinic. Nevertheless, FIP, especially

the noneffusive form, can incubate for months or even years. Cats with FIP usually have a history of stress in the previous few months. Those with effusive FIP are usually taken to their veterinarians within 4 to 6 weeks of arriving in a new home, elective surgery, or a similar stressful situation, whereas cats with noneffusive FIP develop disease after a greater interval. Cats that have spent several years in a single-cat environment are extremely unlikely to have FIP.

Effusive Disease

Cats with effusive FIP have ascites, although very few owners notice the abdominal distention (Fig. 10-2), thoracic effusion (Fig. 10-3), or both. The cat may be bright or dull, anorexic, or eating normally. Abdominal swelling with a fluid wave, mild pyrexia (39° C to 39.5° C [102.2° F to 103.1° F]), weight loss, dyspnea, tachypnea, scrotal enlargement, muffled heart sounds, and mucosal pallor or icterus may be noted. In one survey, FIP accounted for 14% of cats with pericardial effusion, second only to congestive heart failure (28%).[281] Abdominal masses can be palpated, reflecting omental and visceral adhesion, and the mesenteric lymph node may be enlarged.

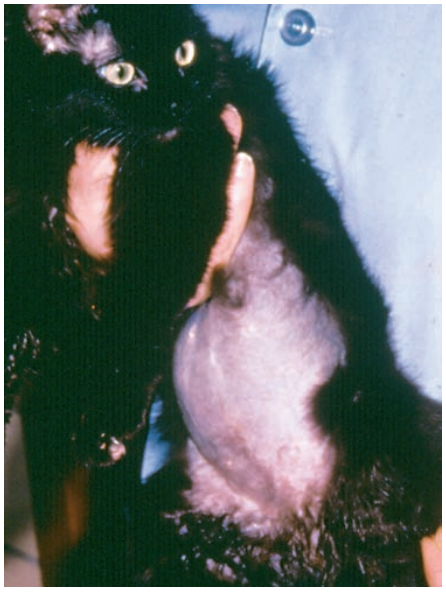

FIG. 10-2 Abdominal distention from FIP effusion. (Photograph by Craig Greene © 2004 University of Georgia Research Foundation Inc.)

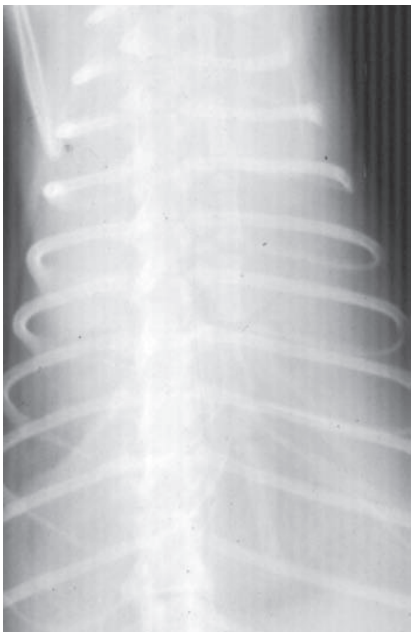

FIG. 10-3 Radiograph of a cat with FIP and thoracic effusion.

Noneffusive Disease

Noneffusive FIP is the more chronic manifestation of the disease, occurring weeks to many months after initial infection and the triggering stress. Signs of noneffusive FIP are usually vague and include mild pyrexia, weight loss, dullness, and depressed appetite. Cats may be icteric. Almost all cats with noneffusive FIP have intraocular lesions. Abdominal palpation usually reveals enlarged mesenteric lymph nodes[162] and may also reveal irregular kidneys or nodular irregularities in other viscera. If the lungs are involved, the cat may be dyspneic, and thoracic radiographs may reveal patchy densities in the lungs.[322]

Ocular Signs. Cats with noneffusive FIP frequently have ocular lesions. The most common ocular sign in FIP is iritis, manifest by color change of the iris. Usually all or part of the iris becomes brown (Fig. 10-4), although occasionally blue eyes appear green. Iritis may also manifest as aqueous flare, with cloudiness of the anterior chamber, which in some cases can be detected only in a darkened room using focal illumination. Large numbers of inflammatory cells in the anterior chamber settle out on the back of the cornea and cause keratic precipitates, which may be hidden by the nictitating membrane (Fig. 10-5). Some cats have hemorrhage into the anterior chamber. If the cat has no sign of iritis, the retina should be checked because FIP can cause cuffing of the retinal vasculature, which appears as fuzzy grayish lines on either side of the blood vessel (Fig. 10-6). Occasionally, pyogranulomata are seen on the retina (see Fig. 10-6); the only other condition likely to produce pyogranulomata on the retina would be mycobacterial infection.[68] The vitreous may appear cloudy. Retinal hemorrhage or detachment may also occur[306] but is more commonly a sign of hypertension. Similar intraocular signs can also be caused by infections with *Toxoplasma* organisms, feline immunodeficiency virus (FIV), feline leukemia virus, or systemic fungi (see Chapter 92).[306]

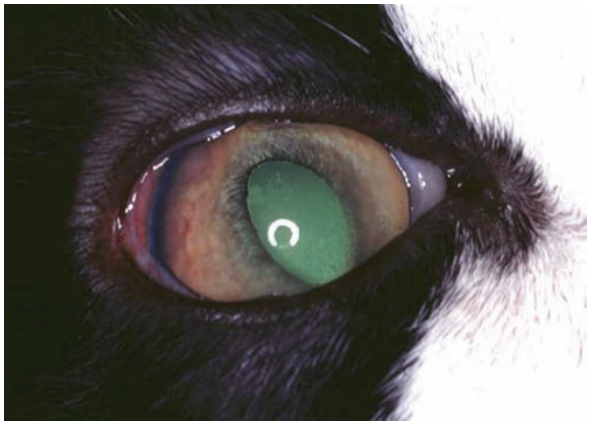

FIG. 10-4 In most noneffusive FIP cases it is possible to find intraocular signs, though the signs can be subtle and a thorough examination required to detect them—such as the iritis seen at the top left of this cat's eye. (Courtesy Diane Addie, Feline Institute, Pyrenees, France.)

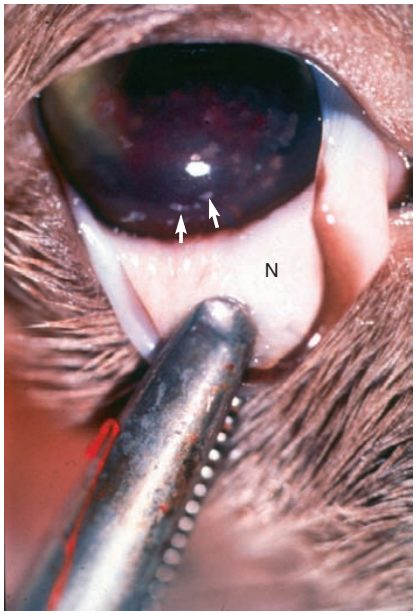

FIG. 10-5 Keratic precipitates on the cornea *(arrows)* in noneffusive FIP. The nictitating membrane *(N)* has been deflected down to enable visualization of the precipitates. (Courtesy Diane Addie, University of Glasgow, Glasgow, Scotland.)

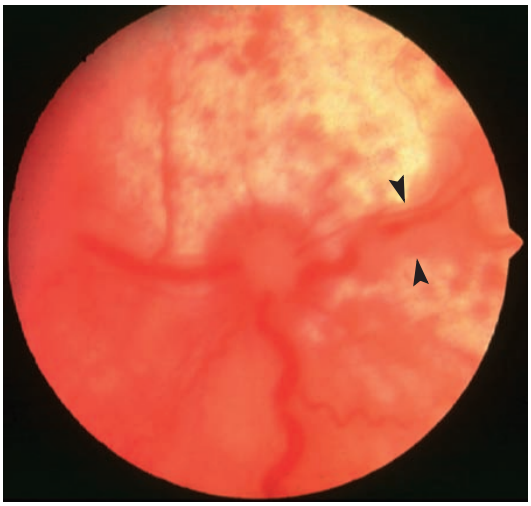

FIG. 10-6 The retina of a cat with noneffusive FIP. The photograph is in focus but appears cloudy because of the high-protein exudate into the vitreous. Cuffing of the retinal blood vessels appears as grayish lines on either side *(arrowheads)*. Retinal blood vessels can be seen disappearing into a pyogranuloma *(P)*. (Courtesy John Mould, Herefordshire, UK.)

Neurologic Signs. In cats with noneffusive FIP, 25% to 33% have neurologic abnormalities.[81] The onset of neurological signs is a poor prognostic indicator, and decerebrate posture (opisthotonos, forelimb extension, hindlimb flexion) a hopeless one.[172,174,257,293] Clinical signs are variable and reflect the area of CNS involvement; the most common clinical sign is altered mental status, then ataxia followed by nystagmus and then seizures.[173] An excellent review of ataxia in the cat was written by Penderis.[257] Ataxia due to FIP can be cerebellar, sensory (spinal or general proprioceptive), or central vestibular but is not likely to be peripheral vestibular.[257] To differentiate central and peripheral vestibular disease: normal postural reactions, ipsilateral cranial nerve VII deficits and Horner's syndrome, and horizontal nystagmus with fast phase away from the lesion side are present in the peripheral vestibular disease. Central vestibular disease may have these signs; however, any additional deficits make it more likely. Discomfort on opening the mouth is a more common feature of peripheral vestibular disease.[257]

When FIP causes nonsuppurative granulomatous meningitis, the signs reflect damage to the underlying nervous tissue: unexplained fever, behavioral changes, seizures, paralysis, incoordination, intention tremors, hypermetria, hyperesthesia, and cranial nerve defects. When the FIP lesion is a pyogranuloma on a peripheral nerve or the spinal column, lameness, progressive ataxia, or paresis (tetraparesis, hemiparesis, or paraparesis) may be observed.[172,173,214,257] FIP is the most frequent cause of spinal cord lesions in cats up to 2 years of age.[194] Cranial nerves may be involved, causing visual deficits and loss of menace response,[172,173] depending on which cranial nerve is damaged. An excellent review of diagnosis and treatment of seizures in the cat has been published by Smith Bailey and Dewey.[293]

Computed tomographic and magnetic resonance imaging (MRI) studies are valuable in the diagnosis of CNS FIP. Occlusion of the aqueduct, causing obstructive hydrocephalus (lateral ventricular width greater than 2 mm) is highly suggestive of a diagnosis of neurologic FIP.[81,172,173,257,262] In a study of 24 cats with FIP and neurologic involvement, 75% were found to have hydrocephalus on gross or histologic postmortem examination.[173] Other diseases such as cryptococcosis, toxoplasmosis, and lymphoma have not been reported to cause hydrocephalus.[173] Isolated fourth ventricle and cervical syringomyelia have also been reported.[172,173] After intravenous contrast medium (gadolinium, gadoteridol), enhancement around the third and fourth ventricles, mesencephalic aqueduct, and brainstem on MRI is highly suggestive of FIP (Web Fig. 10-3).[81,172,257]

Colonic or Intestinal Localization

Occasionally, the primary or only organ affected by FIP granulomas is the intestine. Lesions are most commonly found in the colon or ileocecocolic junction but may also be in the small intestine.[110,327] Cats may have various clinical signs as a result of this lesion—usually constipation, chronic diarrhea, or vomiting.[110,327] Palpation of the abdomen often reveals a thickened intestine. A hematologic finding may be increased numbers of Heinz bodies.

Cutaneous Lesions

Lesions have been described in the skin, always in association with other clinical signs of FIP.[40] These nonpruritic cutaneous lesions have been characterized as slightly raised, well-circumscribed, intradermal papules of approximately 2 mm in diameter over the neck, forelimbs, and lateral thoracic walls.[40,56] Skin fragility similar to that associated with Ehlers-Danlos syndrome has also been reported in a cat with FIP.[321]

Neonatal and Prenatal Kittens

FIP is the second most common infectious cause of mortality in weaned kittens[16,42] but causes no deaths from birth to weaning ("fading kittens"). In the 1970s, FCoV was implicated in various reproductive disorders and in fading kitten syndrome,[287] but the problem was probably due to taurine deficiency, and FCoV is no longer believed to be involved.[16,21] FCoV does not cause infertility.[16] However, FCoV infection does result in stunting of kittens (Fig. 10-7) and increased prevalence of diarrhea and upper respiratory signs.[7]

FIG. 10-7 Uneven litter sizes and stunting of kittens due to FCoV infection. This is an early warning sign that FCoV is endemic in a breeding cattery.

Nondomestic Felidae

FCoV can be an important pathogen for domestic and exotic Felidae. Coronavirus infections have produced chronic weight loss, diarrhea, and anorexia. In a survey of captive felids, more than 50% had positive test results for infection based on fecal PCR and serologic testing for type I and type II coronaviruses.[150] Mortality from FIP has been observed among captive exotic felids, with cheetahs (*A. jubatus*) having the highest risk for disease.* Necrotizing colitis caused by FCoV is a major health problem in cheetahs.[150]

DIAGNOSIS

Coronavirus Enteritis

No specific tests exist for coronaviral enteritis, and FCoV can only be assumed to be the cause of diarrhea in FCoV-seropositive or RT-PCR fecal result-positive cats in which other infectious, inflammatory, or dietary causes have been eliminated. However, a negative fecal RT-PCR result would tend to eliminate coronavirus enteritis as a diagnostic consideration. Biopsy evaluation with conventional staining methods is of limited use because the histopathologic features of villous tip ulceration, stunting, and fusion are nonspecific. FCoV infection can only be confirmed if immunohistochemical or immunofluorescent staining of gut biopsy samples is available.

Feline Infectious Peritonitis

A definitive diagnosis of FIP can often only be made after death, with histopathologic findings consisting of phlebitis or perivascular pyogr anuloma.[166,227,229,287a] In vivo FIP diagnosis is extremely challenging for even the most competent clinician. Even tru-cut biopsy and fine-needle aspirate (FNA) results of the liver and kidney have only 11% to 38% sensitivity in correctly diagnosing FIP.[94]

At most stages of the diagnostic process, it is easier to rule out non-FIP conditions than to be absolutely sure that FIP is involved. The following discussion will parallel the algorithm given in Web Fig. 10-2. The first steps to a diagnosis of FIP are to obtain a history of the cat; review the clinical signs that have given rise to the suspicion of FIP (see Web Fig. 10-2, boxes *2a* and *2b*). The next step involves analysis of the effusion or of the blood; however, if abdominal or thoracic effusion is present, its analysis is more useful and will be discussed first (see Web Fig. 10-2, box *3a*).[109] Nonspecific abdominal ultrasonographic abnormalities can include: peritoneal effusion and abdominal lymphadenomegaly in many cats, and in some cats, hypoechogenicity in the parenchyma of the liver or spleen.[185a]

*References 115, 149, 150, 151, 155, 217, 260.

FIG. 10-8 Positive Rivalta test: one drop of 98% acetic acid is added to 5 mL of distilled water and mixed thoroughly, and a drop of effusion is carefully layered on top. If it disappears and the solution remains clear, the test is negative. If the drop retains its shape, stays attached to the surface, or floats slowly down the tube, the test is positive. (Courtesy Diane Addie, Feline Institute, Pyrenees, France.)

Effusion Analysis

Approximately 50% of cats with effusions have FIP.[200] The FIP fluid may be clear, straw colored, and viscous and because of the high protein content may froth when shaken (Web Fig. 10-4). The effusion may clot when refrigerated. If the sample is bloody, pus-filled, chylous, or foul smelling, then FIP is unlikely,[275] although in rare cases it can appear pink and chylous.[283] The effusion in FIP is classified as a modified transudate in that the protein content is usually very high (greater than 3.5 g/dL), reflecting the composition of the serum, whereas the cellular content approaches that of a transudate (fewer than 5000 nucleated cells/mL). The high protein content of the effusion parallels the increased levels of gamma globulins; thus a low albumin : globulin (A:G) ratio in an effusion is highly predictive of FIP. An A:G ratio of more than 0.8 almost certainly excludes FIP,[288] and with values between 0.45 and 0.8, FIP remains a possibility.[295] An A:G ratio of less than 0.45[296] in an effusion with greater than 3.5 g/dL of total protein and low cellularity, consisting of predominantly neutrophils and macrophages, is highly predictive of effusive FIP.[275] The diseases with similar fluid analyses are lymphocytic cholangitis and occasionally tumors, usually of the liver. Cytology of the effusion, as well as radiographic and ultrasonographic findings, may help to differentiate FIP from neoplasia, cardiomyopathy, and liver disease with portal vascular hypertension.[121,288]

Additional diagnostic tests can be performed on the fluid to help substantiate a diagnosis of FIP. The Rivalta test is a simple, rapid inexpensive point-of-care test for FIP. One drop of 8% acetic acid is added to 5 mL of distilled water and mixed thoroughly, and a drop of effusion is carefully layered on top. If the drop disappears and the solution remains clear, the test result is negative. If the drop retains its shape, stays attached to the surface, or floats slowly down the tube, then the test result is positive (Fig. 10-8). For the Rivalta test result,

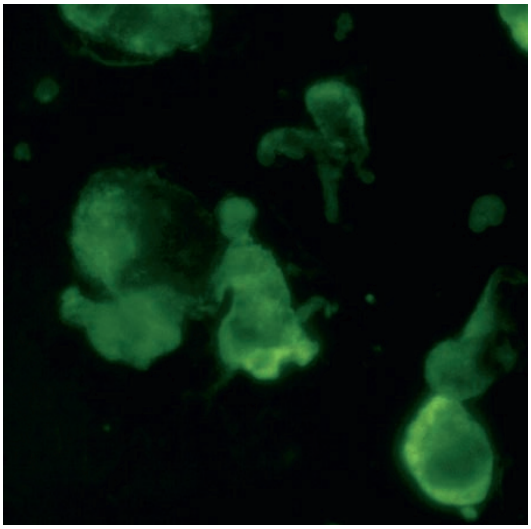

FIG. 10-9 Direct immunofluorescent staining of abdominal effusion showing intracellular coronavirus in a cat with FIP. (Photograph by Wayne Roberts © 2004 University of Georgia Research Foundation Inc.)

the positive predictive value is 0.86, and the negative predictive value is 0.97.[284]

Positive immunofluorescent staining, indicating FCoV-infected macrophages from an effusion, is definitely diagnostic of FIP, but a negative result does not rule it out (Fig. 10-9).[109,233] One difficulty with this test is that often the effusion has few macrophages.

Hematologic and Biochemical Findings

The typical hematologic change in both effusive and noneffusive FIP is lymphopenia.[230] In noneffusive FIP, a nonregenerative anemia (hematocrit [HCT] less than 30%) associated with chronic inflammation is evident (see Web Fig. 10-2). Cats that are constipated from granulomatous colitis have an increase in Heinz bodies in erythrocytes. FIP was the main cause of thrombocytopenia in cats.[175] Serum γ- globulin is a more useful predictive test for FIP than total protein or A:G ratio (Web Fig. 10-5).[109,284,301] The specificity of the diagnosis increases in parallel with value used as a cutoff for increased γ-globulin levels; however, the corresponding sensitivity decreases.[109,284] The serum A:G ratio decreases in FIP because the albumin level remains within reference limits or decreases slightly and globulin levels increase. The total serum protein level is often high. FIP should be suspected when serum protein electrophoresis reveals a polyclonal increase in γ-globulin. Other possibilities for these increases include B-cell lymphosarcoma, multiple myeloma or other plasma cell dyscrasia, or chronic persistent infections such as FIV.[173,193] Other biochemical alterations reflect damage to the organs containing FIP lesions and are not specifically useful for diagnosing FIP. However, they may help the clinician determine whether treatment is worthwhile. Hyperbilirubinemia may be observed and frequently is a reflection of hepatic necrosis. Despite this fact, the alkaline phosphatase and alanine aminotransferase activities are often not increased as dramatically as they are with cholestatic disorders, such as cholangiohepatitis and hepatic lipidosis.

Cerebrospinal Fluid Examination

Cerebrospinal fluid (CSF) analysis is often the most useful for confirming neurologic FIP, but it may be difficult or impossible to obtain a specimen because of the high viscosity of the fluid as a result of protein and inflammatory cell accumulation.[173] The risk of brain herniation is significant in these cases, so care should be taken when performing a CSF tap.[215,257] Analysis of CSF from cats with neurologic signs can reveal spectacularly elevated protein levels.[81,173,215,257,300] However, in one study, CSF total protein was elevated in only 25% of cats with neurologic FIP.[173] Total protein in CSF from healthy cats is less than 0.27 g/L; however, lumbar puncture will give higher total protein levels in CSF than by cisternal puncture.[67] Pleocytosis (5 leukocytes/μL or 100 to 10,000 nucleated cells/μL—neutrophils, lymphocytes, and macrophages) is present in 67% of cats with neurologic FIP. *

Feline Coronavirus Antibody Tests

It has been said that more cats have died because of FCoV-antibody test results than of FIP. Serologic testing can be useful if the laboratory is reliable and consistent and the test results have been correlated intelligently with clinical findings. At times, clinicians have mistakenly equated a positive antibody titer result with a diagnosis of FIP, which is partly the fault of commercial laboratories or kit manufacturers who specifically call their tests "FIP tests," when in fact the tests generally detect only the presence of FCoV itself or FCoV antibodies. False-negative antibody test results can be found if there are numerous virus particles in the sample binding the antibody and rendering it unavailable to the antigen in the test or if the testing is performed too soon after exposure to the virus. Antibodies to FCoV appear 18 to 21 days after infection.[204] Antibody presence without infection may be found early in the neonatal period: MDAs disappear by 5 to 6 weeks of age.

Approximately one third of seropositive cats are actively infected and shedding coronavirus.[10] FCoV antibody titers correlate fairly well with virus shedding.[10,246] However, there are many cats with high titers that do not shed virus, and there are cats with low titers that do shed virus.

Analysis of antibody titers is especially useful when FCoV in a cat population is being controlled by quarantine or when it has been eliminated. When a test is sensitive enough, a seronegative result in a clinically healthy cat means the cat is uninfected. Methodologies and antibody titer results vary among laboratories, but each laboratory should report two titer levels. One is the least significant level of reactivity (or *low* positive titer value) and another is the *high* antibody titer value. High titers have been correlated with a greater chance of FCoV shedding or presence of FIP as demonstrated by confirmation with surgical biopsy or necropsy results.[10,246] The absolute antibody titers mentioned in this chapter are those established by the author's laboratories and should only be used as relative guidelines. When searching for a reliable laboratory, a sample should be divided, stored at −20° C, and sent, without revealing its purpose, to the laboratory in question and an FCoV-referenced laboratory for comparison. See Web Appendix 5 for a listing of some established laboratories for the immunofluorescent antibody test. A final common misconception about antibody titers should be noted. Increasing antibody titers *do not* indicate that a cat is going to develop FIP—the majority of cats with rising FCoV antibody titers subsequently eliminate the virus and have seronegative results again.

FCoV antibody tests based on the 7b protein have been commercially marketed based on data indicating that the less virulent strain, laboratory strain FECV 70-1683, lacked the 7b gene, whereas the highly virulent laboratory strain FIPV 79-1146 had an intact 7b gene.[332] The finding was later found to be a laboratory artifact because FCoVs in cell culture frequently develop deletions in the 7b gene.[119] This gene is not essential for viral replication and seems to be superfluous in the absence of a host. Both cats with FIP and healthy cats

*References 81, 173, 215, 257, 293, 300.

have antibodies to the 7b protein.[152] One study showed distinct genetic differences in the membrane and nonstructural protein 7b genes between FCoV strains from cats with and without FIP.[38] Other investigators have found consistent deletions in the 3c gene within FIPV biotypes.[45,254] The protein encoded by the 3c gene has unknown function but appears to be essential for viral replication in the gut.[45] Whether these genetic discoveries can be exploited to develop a diagnostic test reliably predictive of FIP remains to be seen.

There are 10 major indications for FCoV antibody testing as outlined in Box 10-1 and Table 10-1. The following discussion considers the various types of antibody tests and their uses.

Indirect Immunofluorescence. Indirect FA testing is the gold standard for detection of FCoV antibodies; it is useful because it generates indirect FA titers that correlate well with virus excretion.[10,246] It is clear that seronegative cats, as determined by a *reliable* diagnostic test, do not shed FCoV,[7,10,84] whereas approximately one in three FCoV-seropositive cats do shed virus.[86] Cats with higher antibody titers are more likely to shed virus,[10,84,110,246] although cats with relatively low indirect FA titers of 40 to 80 have a 26% to 39% chance of shedding FCoV.[7,8,106]

Types I and II FCoV and transmissible gastroenteritis virus of pigs can be used in the test.[178] Care must be taken to distinguish fluorescence associated with antibodies to FCoV from nonspecific fluorescence caused, for example, by antinuclear antibodies. These can be present because of other factors such as concurrent infections (e.g., FIV, systemic mycoses), autoimmune disease, recent vaccination, or certain treatments for hyperthyroidism (i.e., thiamazole, felimazole, methimazole). Therefore, inclusion of a negative control of uninfected cells for each serum or plasma is essential.

Enzyme-Linked Immunosorbent Assay. Plate enzyme-linked immunosorbent assays (ELISA) or kinetics-based ELISAs are used in commercial and research laboratories. There are no published refereed veterinary assessments of the sensitivity or specificity of these tests apart from the kinetics-based ELISA.[25] See Web Appendix 5 for the commercial availability of this assay.

BOX 10-1

Indications for Performing Feline Coronavirus Antibody Tests[a]

1. Diagnosis of FIP or coronavirus enteritis
2. Monitoring treatment of a cat with FIP
3. Contact with case of FIP or suspected or known coronavirus excretor
4. Screening a cat before mating
5. Screening a cattery for the presence of FCoV
6. Screening a cat for introduction into a FCoV-free household or cattery
7. Screening a cat before surgery or other stress
8. Screening a cat before giving immunosuppressive drugs

FCoV, Feline coronavirus; *FIP,* feline infectious peritonitis.
[a]Interpretation of FCoV serology in these circumstances is given in Table 10-1.

TABLE 10-1

Interpretation of Feline Coronavirus Serology Results

	FCoV Antibody Test Results	
Reason for Testing	**Positive**	**Negative**
Diagnosis of FIP or coronavirus enteritis	The clinical signs may be related to FCoV infection, but because many cats with diseases other than FIP or coronavirus enteritis will also be seropositive, other parameters must be examined and differential diagnoses carefully eliminated.	Provided the test is sensitive enough, FIP or FCoV are unlikely to be the causes, though occasionally effusive FIPs have so much virus in the effusion that it binds to antibody, rendering it undetectable to some tests.
Monitoring treatment of a cat with FIP	Retest in 2–3 months.	Provided clinical signs and other parameters have returned to normal, it is now safe to discontinue treatment. High doses of glucocorticoids can artificially reduce the FCoV antibody titer.
Contact with case of FIP or suspected or known coronavirus excretor	A cat in this situation would be expected to be seropositive. Monitor antibody titers every 2–3 months until the cat becomes seronegative.	Safe to get another cat
Screening a cat before mating	Either delay mating until seronegative (retest 2–3 months), or use a controlled mating and test queen's feces by RT-PCR on 4–6 occasions; if she is shedding virus, early-wean and isolate kittens.	Safe to proceed with mating
Screening a cattery for the presence of FCoV	Institute regular serotesting every 2 months, separating positive and negative cats.[120] Also use RT-PCR on feces, if possible.	If all cats are seronegative, there is no FCoV in the cattery.
Screening a cat for introduction into a FCoV-free household or cattery	Delay introduction and retest in 2–3 months.	Safe to introduce the cat into the FCoV-free household
Screening a cat before surgery or other stress	If possible, delay stress until seronegative. Retest 2–3 months.	Safe to proceed
Screening a cat before giving immunosuppressive drugs	Examine feces by RT-PCR to establish whether cat is currently infected. Immunosuppression could precipitate FIP—consider alternatives.	Safe to proceed

***FCoV*, Feline coronavirus; *FIP*, feline infectious peritonitis; *RT-PCR*, reverse transcriptase–polymerase chain reaction.**

Point-of Care Antibody Tests. There are at least two FCoV antibody test kits: an ELISA, the FCoV or FIP Immunocomb (Biogal Galed Laboratories Kibbutz Galed, M.P. Megiddo, Israel), and the rapid immunomigration (Speed F-Corona, Bioveto, France; Web Fig. 10-6). The FCoV Immunocomb compared favorably with the gold standard indirect FA test.[12]

Interpretation of Antibody Titer Results. Despite frequent criticism, serologic tests are very useful for identifying cats with suspected FIP, but clinicians should be aware of the limitations of these tests. First, many healthy cats (especially if they are purebred) and cats with conditions other than FIP can have seropositive results. Second, some cats with effusive FIP appear to have low titers or to have seronegative titers because large amounts of virus in their bodies are binding to antibody, making them unavailable to bind the antigen in the serologic test. Although exceptions have been reported,[173,298] cats with noneffusive FIP usually have a high FCoV-antibody titer and rarely have seronegative results; thus, coronavirus serology can usually be used to rule out a diagnosis of FIP in suspected noneffusive cases. The presence of a high FCoV-antibody titer in a sick cat from a low-risk, one- or two-cat household is also unusual; it is a stronger indicator of a diagnosis of FIP than the same antibody titer in a cat from a multicat household in which FCoV is likely to be endemic.

Serologic testing cannot be used alone to diagnose FIP, and the other parameters listed in Web Fig. 10-2 *must* also be considered. Several popular misconceptions regarding interpretation of antibody titers should be addressed. First, clinically healthy cats with FCoV antibodies *do not* have noneffusive FIP. Second, cats with neurologic FIP had higher antibody titers but lower FCoV loads than cats with generalized FIP.[86] Last, seronegativity in diarrheic cats rules out FCoV as a cause; however, FCoV may or may not be a cause of diarrhea in cats with seropositive results.

Effusion. Serologic tests performed on ascites or thoracic effusions yield the same results as when done on blood samples, provided they have high protein concentrations that approximate blood. High FCoV antibody titers in an effusion are 85% specific and 86% sensitive for predicting FIP.[109] As in blood, FIP effusions may appear to have low titers or are seronegative because large amounts of virus in the effusion can bind to antibody, making them unavailable to bind the antigen in the serologic test. One way to resolve this issue is by testing for immune complexes.[109] Alternatively, these effusion samples can be examined further for the presence of virus by quantitative RT-PCR. Usually such cats have huge amounts of virus in the effusion.

Cerebrospinal Fluid. In preliminary studies, measurement of antibodies to FCoV in CSF has been reported to assist in the diagnosis of neurologic FIP.[81] The ratios of serum protein to CSF protein and serum FCoV antibody to CSF-FCoV antibody were always equal to or greater than 1. None of eight controls cats with nonneurologic FIP had anti-FCoV antibodies in the CSF. Unfortunately, these control cats were experimentally infected and had relatively low serum antibody titers to FCoV. Nonspecific leakage of serum proteins into CSF cannot be eliminated: it should be suspected when increased CSF cellularity suggests nonspecific leakage. Adjustment for leakage can be made by comparing a ratio of the CSF-serum titers to another infectious agent (antibody indexing). In a larger studies of natural infection data, with data from corresponding naturally exposed control cats, detection of anti-coronavirus IgG in CSF had a sensitivity of 60% and specificity of 90%, which is of limited clinical use.[34] Because any intracranial hemorrhage in an FCoV-seropositive cat will lead to antibodies in the CSF, FCoV antibodies could be present with conditions other than FIP.

Serologic Monitoring During Treatment. There is very little information published about cats being reevaluated by serologic monitoring during treatment for FIP. FCoV antibody testing is useful at the time of initial diagnosis, but there is little point in monitoring antibody titers more frequently than once every 1 to 2 months once treatment has begun because antibody titers are slow to change. Glucocorticoid or more commonly cytotoxic treatments may suppress the antibody titer, causing it to be artificially low. However, FCoV antibody testing is useful, along with other tests such as α-1 AGP, globulin, HCT, lymphocyte count, and FCoV RT-PCR, in knowing when to discontinue treatment. If a cat has fully recovered, it will have seronegative results; however, if it is only in remission, the antibody titer will likely still be high.

Monitoring Cats after Contact with Virus. Cats that have contacted other cats excreting FCoVs are very likely to have seropositive results, because FCoV is highly transmissible. However, testing can used to compare an initial antibody titer with that of a sample taken 2 to 3 months later, to determine whether or not the antibody titer is declining. If the cat's follow-up antibody titer result is negative (by a reliable, sensitive test), the cat will not develop FIP, it is not shedding FCoV, and it is safe to introduce another cat. Knowing that a cat has positive results for FCoV antibody can enable owners to avoid or reduce stress on the cat in an attempt to prevent FIP.

Screening a Cat before Mating. If the tom and queen cats have negative FCoV antibody results, it is safe to continue with the mating (see the discussion of controlled matings, under Husbandry Measures). If both cats have seropositive results, then viral transmission between the pair is not a problem (although if one cat must travel to the other, the stress of doing so could precipitate FIP). If one has positive results and the other has negative results, then transmission of infection to the noninfected cat is likely, if the cat with positive titer results is shedding virus. Whenever the queen has seropositive results, risk of infecting the kittens is high if she is shedding virus. Therefore, her feces should be tested for virus by RT-PCR on four to six occasions. If the queen is shedding virus, her kittens should be weaned and isolated by no later than 5 weeks of age (Table 10-2).

TABLE 10-2

Protocol for Prevention of Feline Coronavirus Infection in Kittens

Step	Description
Prepare kitten room.	1. Remove all cats and kittens 1 week before introducing new queen. 2. Disinfect room using 1:32 dilution of sodium hypochlorite (bleach). 3. Dedicate separate litter trays and food and water bowls to this room, and disinfect with sodium hypochlorite. 4. Introduce single queen 1–2 weeks before parturition.
Practice barrier nursing.	1. Work in the kitten room before tending other cats. 2. Clean hands with disinfectant before going into kitten room. 3. Have shoes and coveralls dedicated to the kitten room.
Wean and isolate kittens early.	1. Test queen for FCoV antibodies either before or after she gives birth. 2. If queen is seropositive, she should be removed from the kitten room when the kittens are 5–6 weeks old. 3. If the queen is seronegative, she can remain with the kittens until they are older.
Test kittens.	1. Test kittens for FCoV antibodies after 10 weeks of age.

FCoV, Feline coronavirus.

When only one of the pair has positive results, its feces can be tested by RT-PCR to establish whether the infection is current or the antibodies are a remnant of a past infection. If one cat is infected, the mating can be postponed until that cat has stopped shedding virus, usually within a few months.[10] If the breeders are determined to continue, they can do a controlled mating—one in which the cats are put together only for the act of mating. The cats should not be housed together, and most importantly, the uninfected cat should be prevented from contacting the feces of the infected cat by not having access to the litter tray. If the cats are long haired, it can be beneficial to clip the "trousers" to prevent contact with fecal contamination. If the queen is infected, the guidelines on early weaning in Table 10-2 should be followed.

Screening Catteries. It is not necessary to test every cat in a large multicat household, because FCoV is so contagious: In an endemic household, it is expected that 90% of cats would show seropositive results if the virus were present. However, where cats are housed in separate groups, two or three cats from each group should be screened.

Screening a Cat for Introduction into an FCoV-Free Environment. A seronegative cat can be safely introduced. A seropositive cat should not be introduced. It should be quarantined and retested for a titer 2 to 3 months later.

Screening a Cat before Surgery or Other Stress. If the cat is seronegative, it is safe to proceed. However, if the cat is seropositive, it may be at risk of developing FIP. Because only one seropositive cat in three is actively infected, the feces can be examined by RT-PCR to establish whether a cat is currently infected. If the cat is actively infected and it is possible to delay the stress, it should be retested every 2 to 3 months until the cat eliminates the infection. The risk of FIP is greatest in the first 18 months after infection,[18] so simply by waiting, the risk of developing FIP can be decreased. However, if the surgery must proceed, measures can be taken to reduce the stress as much as possible (for example, by scheduling the cat to be admitted for surgery when there is nobody in the waiting room, or by using pheromones to help calm the cat during the procedures).

Screening a Cat before Immunosuppressive Therapy. It is believed that CMI prevents FIP from developing in FCoV-infected cats.[63] Immunosuppressive drugs such as cyclophosphamide, vincristine, or ciclosporin A will suppress CMI. If the cat is seronegative, it is safe to proceed. However, if the cat is seropositive, it may be at risk of developing FIP. Therefore, immunosuppression could precipitate FIP, and if possible alternative treatments should be considered. Because only one cat in three with seropositive results is actively infected, several fecal samples can be examined at weekly intervals by RT-PCR to establish whether a cat is currently infected.

Screening a Blood Donor Cat. If the cat has seronegative results, it is safe to proceed. If the cat has seropositive results, it would be preferable to choose another cat, unless the recipient was extremely unlikely to encounter FCoV in the near future.

Reverse Transcriptase–Polymerase Chain Reaction Testing

PCR is a highly sensitive technique for amplifying and detecting small amounts of DNA (see Chapter 1 and Fig. 1-3). Because FCoV is an RNA virus, a DNA copy must first be made using the enzyme RT. False-negative results can also be generated in the laboratory by the presence of enzymes that destroy RNA (ribonucleases). Real-time or quantitative RT-PCR has been introduced that gives more rapid results and enables quantitation of virus. Viral quantitation may be valuable in discriminating systemic from enteric infections, because the viral load would be much higher in those infections where systemic spread predominates. Viral detection is clinically useful to veterinarians in confirming the presence of FCoV in cats that appear to have FIP but are seronegative and in detecting viral shedding for epidemiologic purposes. Differentiation of enteric FECV and systemic FIPV by genetic methods has been elusive. As stated previously, differences in the 7b and 3c genes have been observed.[38,45,244] These deletions are different in each isolate, currently requiring sequencing to be detected. In addition, there are large variations in viruses from different geographic areas, further complicating the development of a genetically based test.

Blood. Most cats with FIP have negative RT-PCR results in their blood. Viral detection in blood is not a useful prognostic indicator in the healthy cat because FCoV has been detected in the blood of healthy FCoV-seropositive cats.[41,116,291] In addition, the absence of FCoV in the bloodstream does not mean a cat is not going to develop FIP.

Effusions. Viral detection in effusions is useful when it is used concurrently with other diagnostic tests. A positive result is highly suggestive of FIP, but a negative result does not rule it out because many test results on FIP effusions are negative. It could be that centrifuging the cells in an effusion and using them would improve sensitivity.

Cerebrospinal Fluid. FCoV RT-PCR on CSF is not usually useful. Whereas a strongly positive RT-PCR result from a cat with neurologic signs could indicate FIP, the result is often negative in cats with neurologic FIP.[81,86] FCoV has been found in the CSF or brains of two FCoV-carrier cats who died of conditions other than FIP and in one living seronegative cat who had no other clinical indication of FIP.[3] A negative RT-PCR result on CSF does not rule out neurologic FIP.[81,87]

Other Fluids or Biopsy Specimens. RT-PCR on aqueous humor has good specificity for FIP in the anterior chamber.[35] RT-PCR on saliva is not useful in practice because the test results will usually be negative, even in cats with FIP. A positive result from RT-PCR on a conjunctival or nictitating membrane swab for ocular surface cells and tear fluid would be highly indicative of FIP, but a negative result would not rule out FIP. FCoV was detected by RT-PCR in the conjunctivae of 4 of 48 cases of FIP.[187]

Viral detection on an FNA from enlarged mesenteric lymph nodes in cats is a very useful method of diagnosing FIP, because histopathology of an enlarged lymph node is often vague, only describing pyogranulomatous inflammation. However, if RT-PCR on FNA or even tru-cut biopsy is performed on the liver or kidney, it is essential to know that the biopsy came from a lesion within these organs. This is often difficult to achieve if these techniques are done percutaneously, where the lesions are often visualized indirectly via ultrasound, in contrast to direct visualization with laparotomy. With percutaneous methods, it is likely that the result will be negative, especially in a cat with noneffusive FIP with few lesions. Another problem is that a few FCoV-infected cats with diseases other than FIP will have circulating FCoV, and so the blood in the organ being biopsied may yield a false-positive test result. However, when quantitative PCR is used, the amount of virus should be higher in a cat with FIP compared with a cat with another disease.[159]

Feces. Detection or absence of FCoV in the feces is not helpful as a FIP diagnostic or prognostic test, but it is useful in research and in households trying to eliminate FCoV infection. Cats that are chronic FCoV shedders are not at special risk of developing FIP,[15] but the constant source of virus makes it difficult to contain infections in a cattery. However, lifelong FCoV shedders can only be identified by nine consecutive monthly positive RT-PCR fecal tests.[10] RT-PCR cannot measure the viability of the detected organism; therefore, the infectivity of the virus cannot be absolutely ascertained. However, a correlation between strong RT-PCR results and infectivity has been made.[84] There is no need to transport feces to the laboratory on ice. FCoV was still detectable by quantitative RT-PCR in a fecal sample after a month at room temperature and in spite of a fungal growth having appeared on it.[13]

RT-PCR for Messenger RNA. A PCR has been developed that detects replicating coronavirus by targeting the messenger RNA (mRNA).[291] Detection of mRNA in circulating monocytes is evidence that virus is infecting cats systemically and it is replicating. The first developed mRNA RT-PCR yielded positive results in 94% of 49 confirmed cases of FIP and in none of 12 cats with histologically confirmed non-FIP disease.[291] However, in that study, 6% of 326 clinically healthy cats had positive test results. In a second study, 54% of healthy cats had positive test results.[41] The primers for this test detect human DNA, which increases the possibility of obtaining false-positive results. The use of gloves by the veterinary clinician when a blood sample is taken and the use of DNases in the collection media can reduce this risk of false-positive results. One commercially available quantitative FCoV mRNA RT-PCR is available in the United States; however, the methodology and results concerning this procedure have not been published. It is essential to obtain the special transport medium to obtain optimum sensitivity results (see Web Appendix 5).

Antigen Detection in Tissues

Viral detection by direct FA and immunohistochemistry can be applied to effusion, cytologic, or biopsy specimens, but these methodologies must be done by a specialized laboratory.

Immunohistochemical staining, used to demonstrate the presence of virus in the lesions, is the absolute gold standard in FIP diagnosis and is the confirmatory test in cases in which the histologic findings are not typical of FIP.[315,337] However, it is essential that the correct controls be in place (i.e., that a non-FCoV antibody be used as a control because feline tissue is sticky and will often nonspecifically bind irrelevant antibody, such as the conjugated antibody being used to detect the antibody detecting the coronavirus—see Web Fig. 10-7). Lack of these controls will result in false-positive diagnoses of FIP, and laboratories should be consulted to ensure that they are used. Immunohistochemical staining of tissues to detect infected macrophages is a commercially available test (see Web Appendix 6).

In one study of experimentally induced FIP, viral RNA was found in 76% of visceral tissues examined (mediastinal lymph node, spleen, mesenteric lymph node), as compared to 27% of peripheral tissues (popliteal lymph node, cervical lymph node, femoral bone marrow).[55]

FCoV antigen has been detected in swabs made from the nictitating membranes of cats with FIP[132]; however, these results have not been confirmed by other laboratories.[315]

Alpha-1 Acid Glycoprotein and Other Acute-Phase Proteins

In FIP, IL-6 stimulates hepatocytes to release acute-phase proteins. The acute-phase proteins that have so far been examined in FIP are AGP, serum amyloid A, and haptoglobin. Serum amyloid A and haptoglobin are not routinely used in the diagnosis of FIP. High AGP levels are extremely useful to aid diagnosis of FIP[70,226] and for monitoring FIP treatment, because AGP levels decrease rapidly if the treatment is working. The reference range AGP level is 0.1 to 0.48 g/L.[70] A rise in AGP levels was found not only in cats who develop FIP, but also transiently in healthy FCoV-infected cats in contact with the sick cat,[92,228] leading to speculation that the early AGP response could, in fact, be protective against FIP development.[1] Subsequent investigation showed that AGP in cats with FIP was less sialylated than AGP in survivors of FCoV infection.[44] Total sialic acid may help reduce the burden of FCoV in the blood, but the measurement of total sialic acid is not useful in FIP diagnosis.[278]

In using AGP levels to diagnose FIP, it must always be borne in mind that other inflammatory processes, for example an exploratory laparotomy or other neutering surgery,[146] and other infections, such as abscesses or pyothorax or fat necrosis,[224] will increase AGP levels. In conclusion, raised AGP levels cannot be used alone in the diagnosis of FIP but must be used concurrently with other parameters and with consideration of the cat's history and clinical signs.

PATHOLOGIC FINDINGS

The essential lesion of FIP is the pyogranuloma. In effusive FIP, all the surfaces of the abdominal or thoracic contents, or both, can be covered in small (1- to 2-mm) white plaques (Web Fig. 10-8, Figs. 10-10 and 10-11). Few other diseases have similar lesions, although occasionally miliary tumors or systemic mycoses can have similar appearance. In noneffusive FIP, gross pathologic lesions can be much more variable; however, the kidney is frequently affected and should be examined carefully for pyogranulomata in the cortex (Fig. 10-12). In colonic FIP the colon may be thickened and have a gross appearance that is similar to alimentary lymphosarcoma. In some cats, abnormalities are minimal, and a diagnosis can be made only by histologic examination. In the meninges, gross changes are often minimal or consist of hyperemia of the surfaces; however, histologic lesions are characterized by diffuse meningeal infiltration with pyogranulomatous inflammation (Fig. 10-13).

Vasculitis must be demonstrated to diagnose FIP with reasonable certainty. The lesion consists of an arteriole or venule bordered by a

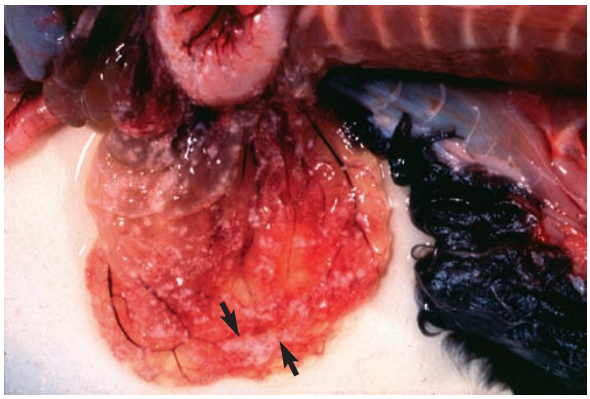

FIG. 10-10 Omentum of a cat with effusive FIP. Note gelatinous appearance and small, white perivascular pyogranulomata *(arrows)* typical of effusive FIP on gross postmortem examination.

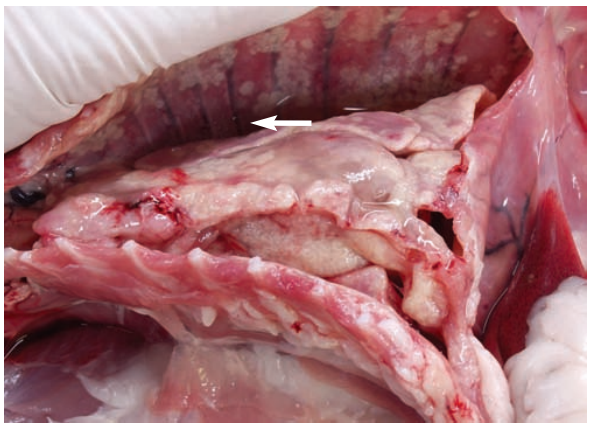

FIG. 10-11 Post mortem of a cat with thoracic effusive FIP, showing a clear, amber effusion *(arrow)*, fibrin on the pleura, and pyogranulomata within the lung. (Courtesy Richard Irvine, University of Glasgow, UK.)

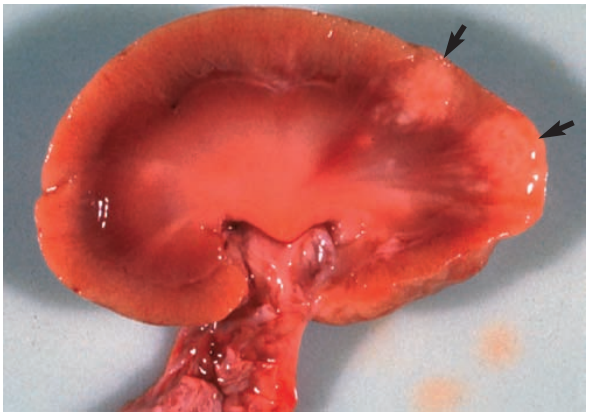

FIG. 10-12 Bisected kidney of a cat with noneffusive FIP showing pyogranulomata *(arrows)*.

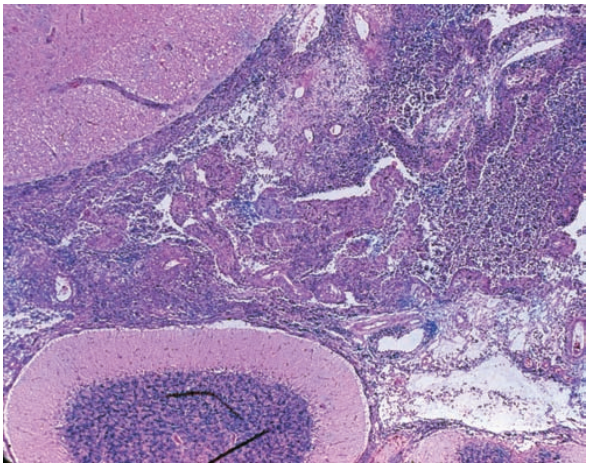

FIG. 10-13 Histopathologic section of surface of cerebellar cortex from a cat with meningeal inflammation from dry FIP (H&E stain, ×100). (Photograph by Craig Greene © 2004 University of Georgia Research Foundation Inc.)

central area of necrosis that is surrounded by a perivascular infiltration of mononuclear cells, proliferating macrophages and lymphocytes, plasma cells, and neutrophils. Immunohistochemistry used to demonstrate the presence of virus in the lesions (see earlier discussion) is the confirmatory pathologic test for suspected lesions (see Web Fig. 10-7).

In cats with coronaviral diarrhea, FCoV can infect the mature columnar epithelium of the tips of the villi of the alimentary tract, resulting in sloughing of the villous tips. FCoV can be demonstrated in the epithelial cells by immunohistochemical staining[9] or immunofluorescence. Mild to moderate villous atrophy may be seen, and villi may be fused.

THERAPY

Healthy Feline Coronavirus–Seropositive Cat

There are three possible reasons for considering treatment of a clinically healthy cat with FCoV-seropositive results. In the *first* case, the cat may be incorrectly diagnosed as having noneffusive FIP because of a false-positive "FIP test" result. Before any treatment is instituted, further testing as outlined previously should be done for confirmation. In the *second* circumstance, treatment might be considered to prevent FIP from developing in a cat that has been in contact with an infected cat. There is no evidence that treatment of a clinically healthy cat with seropositive results could prevent development of FIP. Although it is possible that polyprenyl immunostimulant will prove preventative,[184] more studies are required. Because stress is a common factor in the development of FIP in infected cats,[275] avoiding unnecessary stress such as rehoming, elective surgery, or placement in a boarding cattery may be beneficial. Immunosuppressive drugs such as glucocorticoids should not be given because immunosuppression might precipitate onset of clinical FIP. In the *third* instance, treatment might be considered to stop a healthy FCoV-infected cat from shedding virus. However, no treatment is available that can stop this viral shedding. Various drugs work well against FCoV in vitro, such as ribavirin; however, these drugs do not work in vivo, or else they are toxic to cats.[244]

Ammonium chloride is a frequent constituent of veterinary diets aiming to alter urinary pH. It is also a lysosomotropic agent, inhibiting macrophage invasion by FIPV in vitro.[313] Whether or not a diet containing ammonium chloride would have a protective effect in regard to FIP development is unknown.

Coronavirus Enteritis

There are three manifestations of diarrhea in cats due to FCoV: during primary infection; in persistently infected (carrier) cats; or when noneffusive FIP has caused lesions within the colon. The diarrhea that occurs in some cats with primary FCoV infection is a small intestinal diarrhea that is usually self-limiting within a few weeks. Other causes of diarrhea, such as that caused by *Tritrichomonas foetus*, which is large-intestinal in nature, should be eliminated before FCoV infection is considered responsible. *Typically* the FCoV-induced diarrhea involving young cats living in crowded multicat environments that have seropositive results for FCoV antibody, or in which FCoV has been detected in the feces, can only be treated supportively. FCoV diarrhea in the persistently infected cat is large intestinal, sometimes leading to fecal incontinence. Use of fluid-electrolyte replacement and restricted caloric oral diet with living natural yogurt or with probiotics may be appropriate. No specific antiviral treatment has yet been demonstrated to cure this condition. Some persistently infected carrier cats with diarrhea respond to low doses of prednisolone (0.5 to 1 mg/day).

Clinical Feline Infectious Peritonitis

In general, FIP was deemed incurable, and a diagnosis has resulted in the decision to euthanize. Advances in the understanding of FIP pathogenesis and novel diagnostic tests enable earlier and more accurate diagnosis of FIP, enabling the start of treatment earlier in the disease process, when there is more chance of reversing its course.

FIP is caused not by cytotoxicity of the virus, but by the cat's inflammatory and immune-mediated response to FCoV. Therefore, therapy is aimed at suppressing the inflammatory and immune-mediated responses, often with glucocorticoids. One problem with glucocorticoid therapy is that it affects the immune response nonselectively by suppressing both Th1 and Th2 immune responses. Ideally, treatment should support the Th1 response while suppressing the Th2 response, because there is a hypothesis that a CMI response is beneficial, whereas a humoral immune response is detrimental.[244]

A list of some FIP treatments attempted in the past and possible novel treatments is given next. Although effusive and noneffusive FIP are not distinct diseases, but rather are gradations of the same process, they tend to be differentiated for treatment because the immune reaction is slightly different. See Table 10-3 for suggested therapeutic protocols for each of these conditions. Unfortunately, not all the

TABLE 10-3

Treatment Protocols For Effusive and Noneffusive Feline Infectious Peritonitis

Effusive FIP	Noneffusive FIP
Glucocorticoids: **Dexamethasone:** 1 mg/kg intrathoracic or intraperitoneal injection once only, AND: **Prednisolone sliding dose:** 4 mg/kg/day, PO, for 10–14 days reducing to 2 mg/kg/day for 10–14 days, then 1 mg/kg/day for 10–14 days, then 0.5 mg/kg/day for 10–14 days, then 0.25 mg/kg/day for 10–14 days, then 0.25 mg/kg/e.o.d. and so on, ceasing after complete remission of clinical signs. If, at any point, the cat's condition regresses, go back to the previous dose. **Feline interferon-ω:** 1 MU/kg into the site of the effusion—the abdominal or thoracic cavity or (if not possible) SC, every other day, reducing frequency of treatment to once weekly if remission occurs. **Polyprenyl immunostimulant:** Not advised.	**Polyprenyl immunostimulant:** 3 mg/kg every other day **If Polyprenyl immunostimulant is not available: Glucocorticoids:** **Prednisolone sliding dose:** As for effusive FIP. In addition, for FIP-related uveitis, topical glucocorticoids will be used. **Feline interferon-ω:** 50,000 U per cat PO q 24 hr until AGP, globulins, HCT, lymphocyte count, and clinical signs return to normal. **Diluting feline interferon ω:** Feline interferon ω (Virbagen Omega, Virbac) comes in vials of 10 million units (MU). It is reconstituted with 1 mL of diluent. Ten aliquots of 0.1 mL (1 MU per syringe) are prepared in insulin syringes. Nine of the 10 syringes are placed in the freezer (can be stored up to 6 months). The 10th syringe is diluted with 9.9 mL of sterile 0.9% saline solution to obtain 10 mL of a solution containing a total of 1 MU (100,000 U/mL) of feline interferon-ω. This syringe is stored in the refrigerator at +4°C where it will last up to 3 weeks[3a] (do not freeze diluted interferon-ω; it is unstable). Dose: 0.5–1 mL of this diluted solution (containing 50,000–100,000 units) orally daily, using the syringe without the needle.

AGP, Alpha-1 acid glycoprotein; *E.O.D,* every other day; *FIP,* feline infectious peritonitis; *HCT,* hematocrit; *MU,* million units; *PO,* by mouth; *SC,* subcutaneous.

licensed products are available worldwide. This may affect the choice of regimen.

Glucocorticoids

Glucocorticoids are universally available and inexpensive. Prednisolone is the main immunosuppressant used in the treatment of FIP. They are relatively safe at anti-inflammatory to immunosuppressive dosages and tend to make the cat feel better and stimulate its appetite. Prednisolone suppresses the humoral and CMI response. One cat with noneffusive FIP treated with prednisolone alone survived for 10 months. Prednisolone has the advantage of also being the treatment for lymphocytic cholangitis, which can be mistaken for FIP. When the diagnosis is in doubt and prednisolone is given, a cat with lymphocytic cholangitis has a good chance of recovery, whereas a cat with FIP will not recover.

Prednisolone should never be used in cats with septic peritonitis or pleuritis. Cytologic evaluation of the effusion is important to distinguish FIP fluid from that caused by bacterial or fungal infection. The septic effusion has many more leukocytes, and an attentive cytologist can detect the bacteria or fungi. The dosage is 2 to 4 mg/kg/day given orally, with a gradually reducing dose every 10 to 14 days until the optimal dosage for the cat is determined by continued response to treatment. Cats on immunosuppressive drugs should also be given broad-spectrum antibiotics if secondary bacterial infections arise and possibly given L-lysine (see Chapters 2, 14 and 92) to prevent recrudescence of latent herpesvirus.

Polyprenyl Immunostimulant

Polyprenyl immunostimulant (Sass & Sass, Inc, Oak Ridge, TN) is a mixture of phosphorylated, linear isoprenols that upregulate biosynthesis of Th-1 cytokine mRNAs.[184] It was used successfully in three cats with noneffusive FIP, with survival times of 14, over 26, and 27 months, but it had no beneficial effect on cats with effusive FIP.[184] The dose of 3 mg/kg orally is given two or three times a week until cure (see the Drug Formulary in the Appendix).

Interferon

A good IFN-γ response is thought to confer resistance to FIP.[171] Unfortunately, however, IFN-γ is not available for treating cats, and so treatment must be attempted with recombinant human IFN-α and feline IFN-ω. See Chapter 2 and the Drug Formulary in the Appendix, for further information on these cytokines.

Human Interferon-α. IFNs are species specific. Recombinant human (alpha) IFN (rHuIFN-α) does have some activity in cats,[340] and high doses (10^6 U/kg of body weight) temporarily suppressed disease signs and extended survival in cats with experimentally induced FIP.[340] However, if the cat is still alive after 6 to 7 weeks of this treatment, IFN no longer works because the cat will produce antibodies against it.

Feline Interferon-ω. IFN-ω is a monomeric glycoprotein related to IFN-α and IFN-β but not IFN-γ. It is secreted by virus-infected leukocytes and has antiviral and anti-inflammatory properties. IFN-ω stimulates natural killer cell activity and enhances expression of MHC class I but not class II antigens. MHC in the cat is known as FLA; class I is associated with cellular immunity, whereas class II antigen expression is associated with humoral immunity. IFN-ω is not cross-reactive with IFN-α, so cats that have been treated with and have made antibodies against IFN-α will not neutralize IFN-ω. IFN-ω is acid resistant, so it can be given orally. As with any IFN, it is most effective at the site of the infection.

In the first published report of recombinant feline IFN-ω (rFeIFN-ω, Virbagen Omega, Virbac, France) and prednisolone treatment of FIP, 4 cats of 12 completely recovered and 2 survived 4 and 5 months.[142] However, a later placebo-controlled study involving 37 cats showed no benefit from IFN-ω.[271] The beneficial effect of IFN-ω in treatment of FIP is in question. Although effusive and noneffusive FIP are not distinct diseases, but rather are gradations of the same process, there are two suggested protocols for FIP treatment using IFN-ω (see Table 10-3).

Other Possible Treatments

Thalidomide has anti-inflammatory properties and pushes immune response from Th2 to Th1, so it is theoretically preferable to glucocorticoids in FIP treatment. Thalidomide is not toxic to cats, although its fetotoxicity in pregnant human females has been a concern. Unfortunately, its availability is limited to certain countries. It should *not* be used in pregnant cats. The dosage is 50 to 100 mg once a day in the evening.

TNF-α inhibitors are used to control TNF-α levels that are raised in FIP and contribute to the inflammatory response. Chronic overproduction of TNF-α results in cachexia; therefore it is possible that

TNF-α inhibitors could be used to treat noneffusive and effusive FIP. However, one TNF-α inhibitor, pentoxyfilline, is reported not to be effective.[244] Monoclonal antibodies directed against TNF-α (infliximab) are used in humans with rheumatoid arthritis and Crohn's disease. None have so far been tried in the cat.

Thromboxane synthetase inhibitors (ozagrel hydrochloride, used in humans with asthma), with prednisolone, cured one cat and gave remission for 8 months in a second with effusive FIP.[336] However, this result was unable to be reproduced.[308]

MMP-9 inhibitor (salvianolic acid B) is excreted by monocytes in FIP.[166] MMP-9 inhibitors are zinc-dependent endopeptidases capable of breaking down extracellular matrix proteins. MMP-9 is likely responsible for the leakiness of the blood vessels in effusive FIP. MMP-9 inhibitors may be useful in early effusive FIP but are unlikely to be useful in noneffusive FIP. Although these compounds have not yet been used in cats with FIP, the suggested dose is 10 mg/kg once daily.

5-Hydroxytryptamine receptor antagonist (tropisetron) reduces levels of TNF, IL-1β, IL-6, and prostaglandins.[212] Although this therapy has not yet been used in cats with FIP, the suggested dose is 300 μg/kg once a day.

Compounds with antiviral properties against coronaviruses have been evaluated extensively since the advent of SARS. Results of in vitro screening have indicated antiviral activity in a number of compounds, including some antibiotics and plant lectins.[23,156,324] The plant lectins *Galanthus nivalis* agglutinin, *Hippeastrum* hybrid agglutinin, and *Urtica dioica* agglutinin and the nonpeptidic mannose-binding antibiotic pradimicin A show promise in vitro.[324] The antiviral protein griffithsin specifically binds to the SARSr-CoV spike glycoprotein and inhibits viral entry and has a positive effect on morbidity and mortality in a lethal infection model using a mouse-adapted SARSr-CoV, and also specifically inhibits deleterious aspects of the host immunologic response to SARS infection in mammals.[218] However, there are no successful clinical studies of any of these drugs in cats. See Chapter 2 for an extensive review of antiviral and immunomodulatory drugs.

The Role of Nutrition

It has been recognized that the modern diet of humans, with its omega 6:3 ratio of about 16:1, is hugely different from the 1:1 ratio with which humans likely evolved.[292] It is likely that present-day cats, consuming hugely processed foods containing grain-derived protein that has too much omega 6, have similarly high ratios. Certainly the increase in prevalence of obesity and diabetes mellitus in both people and cats would suggest a similarly disparate nutrition. A high omega 6:3 ratio enhanced proinflammatory cytokine release from monocytes and an increased tendency for monocytes to adhere to endothelium and migrate.[199] Decreasing total polyunsaturated fat content and omega 6:3 ratio in the diet of rats decreased extravasation.[195] Although it has not been tested in controlled studies, giving FIP-cats a high omega 3 supplement might be beneficial. It might also help to prevent the development of FIP in in-contact cats.

Ammonium chloride in vitro reduced FCoV production.[313] Ammonium chloride has been added to feline diets to acidify urine; whether or not such diets would help cure or prevent FIP in cats has not yet been investigated.

Monitoring Treatment and Prognosis

Regardless of which treatment is chosen, it is important to monitor the cat's progress. Regular checks every 7 to 14 days of HCT, globulins, A:G, AGP, lymphocyte count, and the cat's weight serve as indicators of the cat's progress during the first month. Future examinations could be performed at monthly intervals if the cat is improving. It is not worthwhile to measure the FCoV-antibody titer more often than monthly or greater because no discernible difference can be detected within a shorter period. The AGP levels should be the first to decrease if treatment is having a positive effect because AGP is a measure of inflammation. Positive signs also include resolution of effusion; decreasing globulin levels; increasing A:G ratio, HCT, and lymphocytes; the appearance of reticulocytes in blood smears; and weight gain, whereas the opposite changes connote a negative response. If the HCT becomes less than 20% and the anemia is nonregenerative (i.e., no reticulocytes are seen on blood smear examination), the cat should probably be humanely euthanized if its quality of life is impaired. Clearly, if the cat is distressed at any point in the treatment, euthanasia should be considered. Cats with effusive FIP usually survive for only a few days to weeks at best. Cats with noneffusive FIP can survive many weeks or months,[184] although after neurologic signs begin, death usually ensues fairly rapidly.

PREVENTION

Vaccination against FIP

There have been many failed experimental attempts at development of a vaccine against FIP.[244] There is one available vaccine against FIP (Primucell, Pfizer Animal Health, New York) incorporating a temperature-sensitive mutant of the FCoV strain DF2-FIPV, which replicates in the cool lining of the upper respiratory tract but not at the higher internal body temperature.[22,44,45,46] This vaccine, administered intranasally, produces local immunity at the site where FCoV first enters the body—the oropharynx—and also induces a long-lasting CMI response. The vaccine has been available in the United States since 1991 and has been introduced in some European countries. The two concerns about this vaccine are its safety and efficacy.

The safety concern is whether the vaccine can cause ADE. Although some experimental vaccine trials have recorded ADE on challenge, the overwhelming evidence from field studies is that Primucell is safe. None of 582 cats vaccinated with the vaccine and followed for a mean of 541 days showed any ill effects.[266] In two double-blind trials (one with 609 cats[79] and one with 500 cats[264]), the animals were vaccinated with either Primucell or a placebo, and in both trials, fewer FIP-associated deaths occurred in the Primucell-vaccinated group than the placebo group. Clearly, the vaccine afforded protection from FIP and did not cause ADE. Furthermore, immediate side effects from vaccination such as sneezing, vomiting, or diarrhea were not statistically different between the vaccinated group and the placebo group.

Primucell vaccination causes seroconversion, and although it may be at a lower level than that caused by natural infection, it can still cause low positive antibody titers. Cats shed vaccine virus oronasally for up to 4 days. The recommendation for vaccination is to give cats two doses 3 weeks apart from the age of 16 weeks onward. In spite of this recommendation, the vaccine has also been administered to 9-week-old kittens and found to be safe.[141] In these kittens the vaccine did not prevent infection; however, the amount of FCoV isolated from the gut and mesenteric lymph nodes was significantly reduced. The vaccine seems to be safe to administer to pregnant cats and does not affect kitten mortality or reproductive capability in breeding colonies. The vaccine is also safe to administer simultaneously with other vaccines or to cats infected with feline leukemia virus. Annual boosters are recommended. Because mucosal immunity is involved, the duration of immediate IgA protection after natural exposure or vaccination is short in most cats after virus is cleared, and reinfection is possible. Vaccine must be given periodically to maintain this immunity.

The efficacy has been questioned because the vaccine strain is a serotype II coronavirus, and the serotype I coronavirus is more

TABLE 10-4

Protocol for Minimizing Feline Coronavirus Introduction or Spread in a Cattery

Protocol	Description
Reducing fecal contamination of the environment	Have adequate numbers of litter trays (one tray for every one or two cats). Use a nontracking cat litter with some antiviral properties (Addie manuscript in preparation). Declump litter trays at least daily. Remove all litter, and disinfect litter trays at least weekly. Keep litter trays away from the food area. Vacuum around litter trays regularly. Clip fur of hindquarters of longhaired cats.
Cat numbers	Ordinary households should have no more than 8–10 cats. Cats should be kept in stable groups of up to three or four. In rescue facilities, each cat should be kept in single quarters and not commingling with other cats. In a FCoV eradication program, cats should be kept in small groups according to their antibody or virus shedding status: seronegative or nonshedding cats together and seropositive or virus-shedding cats together.
Antibody or virus testing	Incumbent cats should be tested before introducing new cats or breeding. Only seronegative or virus-negative cats should be introduced into FCoV-free catteries. It is safer to introduce seropositive cats than seronegative cats into infected households, but the newcomer and the incumbent cats are still at risk for developing FIP.
Isolation and early weaning	Cat breeders and rescuers of pregnant cats should follow the protocol outlined in Table 10-1.
Vaccination with Primucell	If new cats must be introduced into a household with endemic infection, they should be vaccinated with Primucell (Pfizer Animal Health, New York) before introduction.

FCoV, Feline coronavirus; ***FIP***, feline infectious peritonitis.

prevalent in field isolates. A double-blind trial with 609 16- to 53-week-old vaccinated pet cats was conducted in Switzerland.[79,191] At the start of the trial, 358 cats were seropositive. Up to 150 days after vaccination, the number of cats that developed FIP was not significantly different. However, after 150 days, only one FIP-associated death in the vaccinated group of cats (0.4%) occurred, compared with seven FIP deaths in the placebo group (2.7%).[79] RT-PCR of blood from all of the vaccinated cats that developed FIP showed that virus was present in the cats before the vaccine was administered.[80] Thus, many of the cats in which Primucell appeared ineffective had been incubating FIP before they were vaccinated. Because the vaccine works partly by stimulating local immunity, it is less effective if virus has already crossed the mucous membranes. Obviously, it follows that Primucell is more efficacious in cats that have not been exposed to FCoV (or are seronegative) than in seropositive cats. Clearly, an attempt must be made to prevent kittens from becoming infected with FCoV *before* they are vaccinated.

The efficacy of Primucell based on preventable fraction (see Duration of Immunity and Antibody Measurement, Chapter 100) has been reported to be 50% to 75%. In a survey of 138 cats from 15 cat breeders, in which virtually all of the cats were seropositive, no difference in FIP-associated deaths was found between the vaccinated group and the placebo group.[79] The manufacturers do not specify that FCoV antibody testing should precede vaccination. However, because the vaccine does not work in a cat that is incubating the disease, FCoV antibody testing is beneficial. In addition, the vaccine causes seroconversion and low antibody titers; therefore, testing before vaccination is advisable. Primucell is designated noncore by the American Association of Feline Practitioners (AAFP) and the Advisory Board on Cat Diseases (ABCD).[4,269] Although vaccination is unlikely to prevent FIP in purebred kittens unless they have been vaccinated before exposure, the vaccine is the only option to reduce the prevalence of FIP in cats entering endemically infected shelters or boarding catteries.

The next generation of FIP vaccines could involve genetically modified FCoV. Experimental vaccines in which accessory genes 3abc and 7ab were deleted protected cats against challenge in the laboratory.[107] Curiously, deleting 3abc gave good protection, and deleting 7ab gave some protection, but deleting all of the genes failed to protect.

Feline Infectious Peritonitis Prevention for Cat Breeders

Purebred cats are at greater risk of developing FIP than are nonpurebred cats.[83,259,274,297,344] Even in breeding catteries where FIP has not occurred, problems will arise in the kittens, such as diarrhea and upper respiratory signs.[7] Stunting and uneven litter sizes (see Fig. 10-7) at time of vaccination are excellent warnings that FCoV is endemic and indicate that intervention is needed before disaster strikes.

The elimination of FCoV from purebred cats could be hampered by what has been termed the "ostrich syndrome" among cat breeders. This syndrome is a preference not to know the FCoV status of their cats.[344] However, elimination of FIP from purebred cats should be a goal of conscientious breeders. Good hygiene and cattery design are essential for minimizing the level and spread of FCoV. A protocol for use in catteries is presented in Table 10-4.

Husbandry Measures

Uninfected cats should be separated from FCoV-infected cats, and new cats should be quarantined before being allowed to mix with existing cats. FCoV infection is maintained in a household or cattery by continual cycles of infection and reinfection.[15,84,85] There are now commonly available RT-PCR tests that can detect FCoV in feces, so that it is possible to establish which cats are shedding FCoV and to separate them from cats that are not shedding FCoV. Virus shedding usually continues for 2 to 3 months or longer,[10] so testing feces once a month is adequate. Cats that shed FCoV for 9 months or more are likely to be lifelong carriers of the virus, although one cat known to the author (DA) ceased shedding virus after 5 years. By repeat testing and separation of shedding and nonshedding cats, it is possible to eliminate FCoV from a multicat pet or breeding household.[120] Quarantine and testing of new arrival cats will prevent FCoV from being introduced into such a household.

Controlled matings can be used when one cat is infected with FCoV and the other cat is uninfected, because direct transmission of FCoV is not a problem between healthy infected cats.[3] In controlled matings, the cats are put together only to mate; they do not share a litter tray (see the earlier discussion of screening a cat before mating, under Diagnosis).

Early weaning and isolation of kittens, more than any other factor, determines whether they become infected with FCoV.[6,7] Kittens of FCoV-shedding queens should be protected from infection by MDA until they are at least 5 to 6 weeks old. A protocol for the prevention of FCoV infection in kittens is presented in Table 10-2. When reliable serologic tests are available, kittens should be tested when older than 10 weeks to ensure that isolation and early weaning have been effective. Infected kittens younger than 10 weeks may not yet have seroconverted.[7] Some feel that preventing infection of kittens is too difficult,[244] yet it was done successfully by breeders of 12 litters in ordinary houses, with no special facilities.[7] Even isolating each litter with its respective queen considerably reduced their chances of becoming infected compared with allowing the kittens free access to the whole household.[6,7]

Feline Infectious Peritonitis Prevention in Rescue Shelters

A combination of vaccinating cats with Primucell before they enter shelters or as soon as they enter,[264] excellent hygiene, barrier nursing practices, and stress reduction is necessary to prevent FIP in shelter situations. Cats should be kept away from dogs not only to reduce stress, but also to prevent contact with CCoVs.[268]

PUBLIC HEALTH CONSIDERATIONS

Humans cannot become infected with FCoV or develop FIP. Human coronaviruses 229E and OC43, which are widely prevalent in the human population, cause the "common cold," and do not pose a risk to cats. SARSr-CoV has been experimentally inoculated into cats in the laboratory and caused inflammation of the tracheobronchial tissues and associated lymph nodes.[323] Natural infections with the SARSr virus have been reported in the civet cat, which is a nonfeline carnivorous species.

CHAPTER 11

Feline Leukemia Virus Infection

Katrin Hartmann

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Feline leukemia virus (FeLV) infection occurs worldwide.[62,93] For many years after its discovery, FeLV was considered to (1) be the principal scourge in cats, (2) account for most disease-related deaths in pet cats, and (3) be responsible for more clinical syndromes than any other single agent.[375]

FeLV was first described in 1964 by William Jarrett and co-workers, when virus particles were seen budding from the membrane of malignant lymphoblasts from a cat with naturally occurring lymphoma (Figs. 11-1 and 11-2).[218,219] The virus was shown to produce a similar tumor when experimentally injected into healthy cats and thus was proven to be capable of transmitting neoplasia. Although clusters of lymphoma cases occurring in households had always been observed, it was not until the discovery of FeLV that an infectious etiology was finally proven. After this discovery, it was assumed for many years that all hematopoietic tumors in cats were caused by FeLV, independent of whether the cats were found to be FeLV-positive.[146] Later, it had been estimated that at least approximately one third of all cancer deaths in cats were caused by FeLV, and an even greater number of infected cats died of anemia and infectious diseases caused by suppressive effects of FeLV on bone marrow and immune system.[62] However, today these assumptions are being reconsidered because the prevalence and importance of FeLV as a pathogen in cats are decreasing, primarily because of testing and eradication programs and routine use of FeLV vaccines. It is currently accepted that tumor-causing factors other than FeLV play more important roles, specifically in older cats.[279]

ETIOLOGY

FeLV, a γ-retrovirus of domestic cats, is a member of the Oncornavirus subfamily of retroviruses. It contains a protein core with single-stranded RNA protected by an envelope. FeLV is an exogenous agent that replicates within many tissues, including bone marrow, salivary glands, and respiratory epithelium. If the immune response does not intervene after initial infection, FeLV spreads to the bone marrow and infects hematopoietic precursor cells. All retroviruses, including FeLV, rely on a DNA intermediate for replication. The single-stranded RNA genome is reversely transcribed into DNA, which is randomly integrated into the host's cell genome (the integrated DNA is called "provirus") with the help of an integrase (Fig. 11-3). After reverse transcription, synthesis of viral proteins occurs with assembly of the virions near the cell membrane and budding from the cell (see Fig.

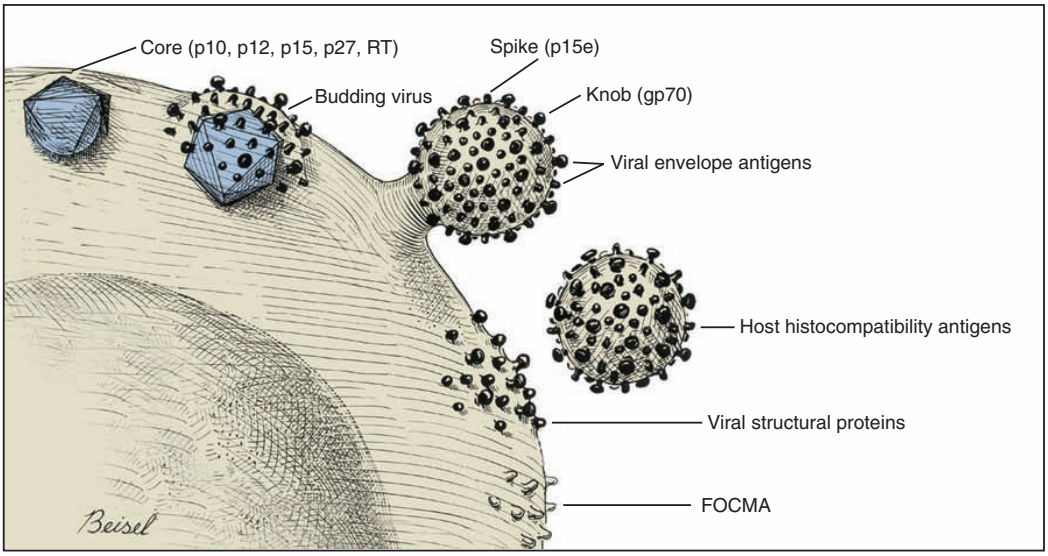

FIG. 11-1 Production and release of virus from a feline malignant cell. Viral envelope antigens can have a spike or knob shape. Host histocompatibility antigens may appear on the virus as the virus buds from the cell membrane. Viral structural proteins may appear on the host cell. Virus replication can also occur in nonmalignant cells. *FOCMA*, Feline oncornavirus cell membrane antigen. (Art by Dan Beisel © 2004, University of Georgia Research Foundation Inc.)

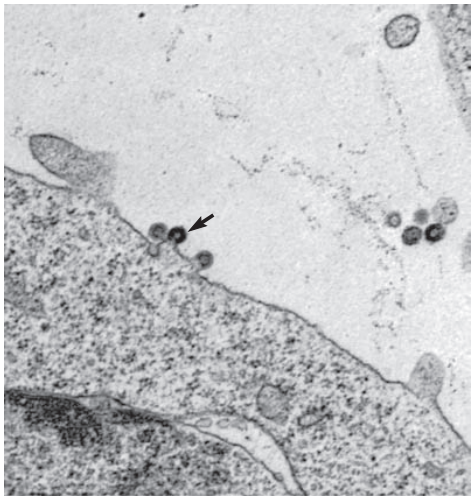

FIG. 11-2 Ultrastructural view of FeLV budding from cell surface *(arrow)*. (Courtesy SmithKline Beecham Animal Health, Exton, PA.)

11-1). Infection of a cell by a retrovirus does not usually lead to cell death. Once the provirus is integrated, cell division results in daughter cells that also contain viral DNA. The ability of the virus to become part of the host's own DNA is crucial for the lifelong persistence of the virus after bone marrow infection. Consequently, every infected cell has to be recognized and destroyed to "cure" an infection. Once the pool of hematologic and immune stem cells becomes infected, true elimination of the virus becomes impossible.[48,187,257]

Virus Origin

Both exogenous (foreign, "pathogenic") and endogenous (inherited, "nonpathogenic") retroviruses occur in cats.[347a] Pathogenic exogenous viruses that can be transmitted horizontally from cat to cat include FeLV, feline immunodeficiency virus (FIV, see Chapter 12), and feline foamy virus (also known as syncytium-forming virus, see Chapter 15), which is widespread but has a low pathogenicity.

On the basis of similarities in nucleotide sequences, it is likely that FeLV evolved from a virus in an ancestor of the rat. It is likely that this event took place in the late Pleistocene up to 10 million years ago in the North African desert. Ancestral rats and cats roamed freely, and the virus was transmitted to cats through ingestion or a rat bite. The initial spread of FeLV among cats might have been inhibited by the aridity of the North African desert.[29]

FeLV is divided into several subgroups (based on the genetic map), but only subgroup FeLV-A is infectious and transmitted from cat to cat. The other subgroups (e.g., FeLV-B and FeLV-C) are not transmitted from cat to cat under natural circumstances but can be generated de novo in a FeLV-A-infected cat by mutation and recombination of the FeLV-A genome with cellular genes or genes from endogenous retroviruses in the cat's genome. The feline sarcoma virus (FeSV) also is a recombination of the FeLV-A genome with tumor-associated cellular genes (proto-oncogenes) and likewise is generated de novo in a FeLV-A-infected cat.

Certain endogenous, nonpathogenic retroviruses (e.g., enFeLV, RD-114 virus, MAC-1 virus) are normally present in the genome of the cat population and inherited by transmission from mother to kitten through germline. These endogenous fractions of proviral DNA (also called "proviral sin") cannot produce infectious virus particles themselves. They are present in every feline cell but not replicating. Their main relevance relies on the fact that these DNA fractions can potentially recombine with FeLV-A DNA in cats with FeLV-A infection and thus increase the pathogenicity of FeLV-A. EnFeLV is thought to have originated hundreds of thousands of years ago from cats that had eaten mice viremic with a murine leukemia virus (MuLV) that was able to incorporate its genome into the germline cells of the predator. This MuLV was then inherited by all the feline offspring. The enFeLV genome is not complete and, therefore, is not competent to replicate by its own.[409] The amount of enFeLV varies between different breeds of cats, including the wild cat (*Felis silvestris*), suggesting that this exposure to MuLVs is a continuing

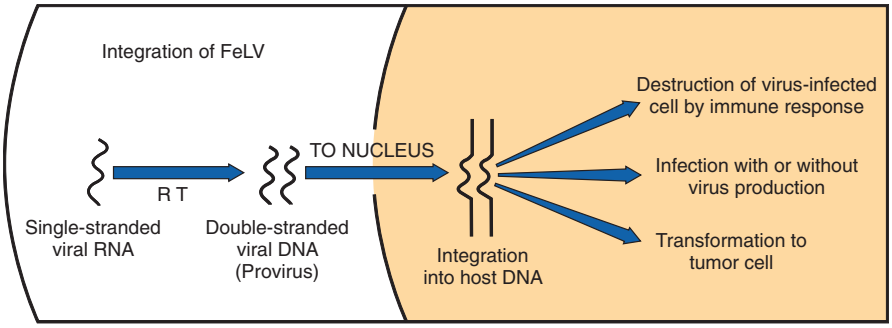

FIG. 11-3 Formation of FeLV and integration into cells. *RT*, Reverse transcriptase.

TABLE 11-1

Feline Leukemia Virus Subgroup[a]

Viral Subgroups	Frequency of Isolation in FeLV-Positive Cats	Associated Disease	Comparison by Species of in Vitro Replication
A	100% in infected cats, mildly pathogenic but highly contagious, mildly cytopathogenic	Immunosuppression and other FeLV-associated diseases, replicating and contagious	Cat, rabbit, pig, mink, human
B	Occurs with subgroup A in 50% or more of cats with neoplastic disease (lymphoma)	Hematopoietic neoplasia, nonreplicating and noncontagious, virulent in recombination with subgroup A	Cat, dog, cow, hamster, pig, human
C	Rarely isolated, mainly in cats with nonregenerative anemia	Nonregenerative anemia and erythremic myelosis, nonreplicating and noncontagious, virulent in recombination with subgroup A	Cat, dog, guinea pig, human

Modified from Jarrett O. 1990. Feline leukemia virus subgroups, pp 473-479. *In* Hardy WD, Essex M, McCleland AJ (eds), Feline leukemia virus. Elsevier, New York; Nakata R, Miyazawa T, Shin YS, et al. 2003. Reevaluation of host ranges of felline leukemia virus subgroups. *Microbes Infect* 5:947-950.

phenomenon,[347a,426] and an association between enFeLV loads and FeLV-A replication but not with outcome of FeLV-A infection was demonstrated.[425]

RD-114 is of primate origin and is most closely related to an endogenous baboon retrovirus and only distantly related to FeLV. It is thought to have originated hundreds of thousands of years ago from an ancestor cat that had preyed on an early primate infected with this RD-114 virus.[23] RD-114 is replication competent. Although no evidence shows pathogenicity of or any immune response to RD-114 virus in cats, it may play some role in normal fetal differentiation.[58,62,434] It also appears important to monitor RD-114 virus production in feline cell lines used for biological products as substrates, and assays to screen for RD-114 infection in cell culture have been developed.[383]

Feline Leukemia Virus Subgroups

FeLV exists in several subgroups that are mainly defined by host cell spectrum, on the basis of their ability to replicate in nonfeline tissues, interference testing, and virus neutralization (Table 11-1). The three most important FeLV subgroups are FeLV-A, FeLV-B, and FeLV-C, all immunologically closely related. Other less important subgroups have been described, including subgroup T, which is highly cytolytic for T lymphocytes and causes severe immunosuppression.[24,250,251] A particular "FeLV feline acquired immunodeficiency syndrome" (FAIDS) is composed of FeLV-A virus and highly immunopathogenic variants that infect CD4+ and CD8+ lymphocytes and B lymphocytes in blood, lymph nodes, and myeloid cells.[354] This widespread proliferation greatly impairs the immune response.

Only FeLV-A is contagious and passed horizontally from cat to cat in nature. The other subgroups evolve de novo in a FeLV-A-infected cat by mutation and recombination between FeLV-A and cellular or endogenous retroviral sequences contained in normal feline DNA. Subgroup B originates from recombination of FeLV-A with enFeLV. Subgroup C is less common and is the result of mutations in the *env* gene. It has been suggested that FeLV-C arises in FeLV-A-infected cats through intermediates that are multitropic in their receptor use.[392] Replication of FeLV-B and FeLV-C is only possible with the help of FeLV-A, because important genomic sequences are replaced in these recombinant viruses. Proposed FeLV-A helper functions include enhanced replication efficiency, immune evasion, and replication rescue for defective FeLV-B and FeLV-C virions. However, in certain experiments, it was possible to induce replication without FeLV-A. In newborn specific-pathogen free kittens, experimental FeLV-B or FeLV-C infection has been established without presence of FeLV-A.[27,387] Nevertheless, all naturally infected cats carry FeLV-A either alone or in combination with FeLV-B, FeLV-C, or both. Thus, if antibodies against subgroup A are produced, the cat is protected against any FeLV infection.

Pathogenicity of FeLV-B and FeLV-C, in combination with FeLV-A, is higher than that of FeLV-A alone.[374] However, in one experiment, infection of FeLV-A in combination with FeLV-B under experimental conditions was associated with an attenuated infection compared to infection with FeLV-A alone when inoculation of different subgroups was performed simultaneously.[344] Different properties of the envelope proteins in the various subgroups have been shown to be the major pathogenic determinant, but the mechanisms by which envelope differences influence pathogenesis are not well understood.[317] FeLV-B is commonly associated with malignancies; FeLV-C is mainly associated with nonregenerative anemia. In experimental infections, a FeLV-B strain (Rickard strain) caused lymphoma in nearly 100% of kittens by 1 year of infection, whereas FeLV-C isolates repeatedly produced fatal nonregenerative anemia.[338] FeLV-B has been associated with a majority of cats with thymic lymphomas.[4]

Feline Leukemia Virus Genome and Proteins

FeLV is a typical retrovirus, containing single-stranded RNA that is transcribed by the enzyme reverse transcriptase (RT) into DNA, the so-called provirus that is subsequently integrated into the cellular genome. The gene sequence contains long terminal repeats (LTRs), which are repeated sequences that have regulatory function and control expression of the other viral genes but generally do not code for a protein product. From the 5′ to the 3′ end, the gene order is LTR-*gag*-*pol*-*env*-LTR. LTR regions play a critical role in tissue tropism and pathogenic potential of the viruses. Within the LTRs, recurrent enhancer sequences or upstream region enhancers (UREs) are frequently found in cats with myeloid leukemias and thought to play some role in oncogenesis.[298,325] Of the UREs, the U3-LTR of FeLV upregulates specific cellular genes in an integration-independent way. The U3-LTR region does not encode a protein but instead makes a specific RNA transcript. It was demonstrated that FeLV U3-LTR upregulates the NFκB signaling pathway via activation of Ras-Raf-IκB kinase and degradation of IκB, providing new explanations of LTR-mediated cellular gene transactivation that might play a role in oncogenesis.[2] The *gag* (group-associated antigen) gene encodes the internal structural proteins, including p15c, p12, p27, and p10 (Table 11-2). The *gag* protein p27, which is routinely used for diagnosis of FeLV infection, is produced in virus-infected cells in amounts exceeding what is necessary for assembly of new virus particles. Thus, p27 is abundant in the cytoplasm of individual infected cells and also in the blood of infected cats, which is why most available immunochromatographic tests, such as the enzyme-linked immunosorbent assay (ELISA) and immunofluorescence assays, are designed to detect this protein, in blood or intracellularly, respectively. Free p27 not only circulates in blood but is shed in tears and saliva, where it also can be detected. The *pol* (polymerase) gene specifies the viral enzyme RT, which is responsible for synthesis of DNA on the RNA template. The *env* (envelope) gene encodes the envelope components gp70 and p15e. The *env* protein gp70 defines the virus subgroup and appears to be important for inducing immunity. Antibodies to gp70 are subgroup-specific and result in neutralization of the virus and immunity to reinfection. Thus, gp70 is important in natural resistance and, therefore, as a target for vaccine production. The transmembrane protein p15e is thought to interfere with host cell immune responses, thus facilitating viral persistence.

EPIDEMIOLOGY

In nature, FeLV has been reported to mainly infect domestic cats. There is evidence, however, that some wild felids are susceptible, and many studies have focused on the presence of FeLV in wildlife species.

Host Range

In vitro, FeLV can replicate also in nonfelid cell lines (see Table 11-1). For example, FeLV-B replicates in cells derived from cats, dogs, cows, pigs, hamsters, monkeys, and humans; FeLV-C replicates in cells of cats, dogs, guinea pigs, and human beings.[214,217,386] It was thought that FeLV-A only replicates in cat cells in vitro, and that infection in vivo that always requires FeLV-A, therefore, cannot occur in nonfelids. However, it has been found that two independent FeLV-A isolates from United Kingdom and United States also have infected various nonfeline cell lines including cells from human beings, rabbits, pigs, and minks.[322] Although malignant transformations do not occur in nonfelid cell cultures,[272] experimental FeLV infection with development of lymphomas could be induced in young dogs and marmosets.[367] In experimental infections with FeSV, fibrosarcomas also could be produced in nonfelids in vivo.[8] However, no reports have been made on natural transmission of FeLV to nonfelids.

Documentation of FeLV in nondomestic felids, however, becomes more and more common, and FeLV appears to be enzootic in some wild felids. Introduction of FeLV into free-living and captive nondomestic felid populations has serious consequences for their health and survival. FeLV infects small wildcats including *F. silvestris*[71,460] and European and Iberian lynxes.[278,306] FeLV also has been detected in the Florida panthers (*Puma concolor coryi*) and causes severe problems in this species, in which vaccination programs now have been instituted.[37,70] A multicentric T-cell lymphoma associated with FeLV infection was found in a captive Namibian cheetah (*Acinonyx jubatus*).[297] FeLV was also detected in an 11-month-old captive-bred male neutered bobcat (*Felis rufus*) showing signs of lethargy, anorexia, neutropenia, lymphopenia, and nonregenerative anemia.[405] Although in one study, FeLV was not found in 12 ocelots (*Leopardus pardalis*) in Barro Colorado Island,[116] FeLV proviral DNA was detected in one male captive ocelot and one female little spotted cat (*Leopardus tigrinus*) in a wildlife center in southern Brazil.[139] There is no evidence of FeLV infection in African lions (*Panthera leo*) or Asian lions (*P. leo persica*).[90,156a,358,359]

TABLE 11-2

Summary of Genetic Map and Function of FeLV Proteins[a]

Gene	Location	Type	Function
gag	Core		Basis for antigen tests (ELISA/ICGA and IFA), role in immune complex disease, and cytotoxic effects
		p15c	Matrix protein
		p12	Unknown
		p27	Capsid protein used for antigen testing
		p10	Nucleocapsid protein
pol	Core	RT	Enzyme copying viral RNA into complementary DNA strand (“reverse transcription”)
env	Envelope	gp70	External surface unit; type-specific antigens FeLV-A, FeLV-B, FeLV-C; responsible for neutralizing or protective antibody production
		p15e	Transmembrane protein; role in immunosuppression

ELISA, Enzyme-linked immunosorbent assay; ***env***, envelope; ***FeLV***, feline leukemia virus; ***gag***, group-associated antigen; ***gp***, glycoprotein; ***ICGA***, immunochromatography assay; ***IFA***, immunofluorescent antibody; ***P***, protein (number is molecular weight in kilodaltons); ***pol***, polymerase; ***RT***, reverse transcriptase.

[a]As listed in chart, genes are located from the 5′ to the 3′ end with long terminal repeat (LTR) sequences at each end.

Prevalence

FeLV infection exists in domestic cats worldwide. Prevalence studies have focused on the detection of FeLV mainly in third-world countries or on remote islands, where the prevalence of virus infections in cats was unknown. In these studies, FeLV has been detected almost everywhere.[31,54,72,284,307] Only cats on Grenada Island, West Indies, and Isabela Island, Galapagos, were free of FeLV infection.[91,261] In contrast to FIV infection, in which the prevalence varies significantly, the FeLV infection rate of free-roaming cats is similar throughout the world, ranging from 1% to 8% in healthy cats.[20,126,264,269,410] Prevalence is as high as 38%, if only sick cats are included in the surveys.[15,157,257] Originally, certain diseases, such as lymphoma, were associated with very high rates (up to 75%) of FeLV infection. Cats that have positive test results for FeLV have become less common because the overall prevalence of FeLV infection has decreased, presumably as a result of control measures.

A number of reports document that the overall rate of FeLV infection is decreasing. For example, the Tufts Veterinary Diagnostic Laboratory, where approximately 2000 serum samples are tested yearly for FeLV antigen, reported a decrease from 8% in 1989 to 4% in 1995.[61] In Germany, a steady decrease in FeLV prevalence from 6% to 1% was observed when investigating the FeLV infection rate from 1993 to 2002.[126] Studies report a prevalence of 2.3% to 3.3% in North America, 0% to 2.9% in Asia, and 1.0% to 15.6% in Europe.* There are a number of possible explanations for the decrease in prevalence. It is most likely the result of test and removal programs at breeding facilities, the practice of testing cats at animal shelters before adoption, and the widespread use of vaccination. None of the available vaccines have been shown to provide 100% protection against progressive infection, but the common practice of vaccination likely has had an impact on the prevalence of FeLV. Although vaccination contributes to the decrease, epidemiologic studies suggest that testing and removal practice is more effective than vaccination.[380] The first vaccine was introduced in 1985, but the observed decline in the overall infection rate began before this time.[257]

Many deterministic models have been constructed to predict the dynamics of FeLV in cat populations. These models predicted that FeLV dynamics depend on the size of the host population and the relationship between host density and the pattern of contacts of individual cats. They found no threshold population size for virus persistence in large populations, but the possibility of FeLV extinction in small populations.[118] Models take into account that cat populations can be connected to each other by dispersal of individuals, which favors roaming of cats and spread of disease.[117] These models explain the geographic discrepancies of FeLV prevalence. Although the absolute number of pet cats is remarkably higher in Northern European countries (e.g., 10 million in the United Kingdom, 8 million in Germany, 10 million in France) than in southern European countries (e.g., 4 million in Spain), living conditions differ considerably. Hence, the higher number of free-roaming cats in southern Europe increases the contact rate in these countries, which, as a consequence, increases the overall prevalence of FeLV infection.[117] Additionally, discrepant results in FeLV prevalence are based on the health status of the cats under consideration.[269] Whenever only clinically healthy cats are included, the prevalence is noticeably lower than in surveys of sick cats.[15,201,441]

Certain risk factors contribute to a higher prevalence. Prevalence of FeLV is higher in cats that are allowed to roam outside,[126,264] because direct contact is required for transmission. In a study in the United States, antibody prevalence (which predicts exposure) was clearly related to the time spent outdoors and the degree of exposure to other cats. Of cats in a study in Boston and Detroit, of which many were allowed to roam outside, 63% and 47% had positive serum FeLV antibody test results, respectively, whereas only 5% of New York cats that were primarily confined to high-rise apartments had FeLV-specific antibodies.[338] One study looked into risk of disasters on FeLV infection rates among cats exported from the 2005 Gulf Coast hurricane disaster area, but could not demonstrate an increase in infection rates in this situation.[262] Risk groups for FIV and FeLV infections are only slightly different. Although fighting, free-roaming, intact male cats are still considered mainly at risk for acquiring FIV infection, the same risk factors also facilitate FeLV infection. FeLV can no longer be considered primarily an infection of "social cats," although FeLV is easily spread through social contacts. In earlier studies, FeLV infection rate was found to be almost equal in male and female cats. In one older study, 733 feral free-roaming cats in Raleigh, North Carolina, and 1143 feral free-roaming cats in Gainesville, Florida, were tested for FIV and FeLV infection, and prevalence of FeLV infection was not significantly different between males (4.9%) and females (3.8%).[252] However, two more contemporary studies, in the United States and Germany, found a significantly higher risk of FeLV infection among male cats.[126,264] Although FeLV transmission commonly occurs between infected queens and kittens and among cats living in prolonged close contact, it seems that aggressive behavior, a common male attitude, plays a greater role than previously reported.[129] Thus, the common opinion that FeLV was a disease of "friendly" cats should be reconsidered. This is also supported by the findings that cats exhibiting aggressive behavior have a higher risk of FeLV infection,[127] and more than 8% of cats examined by veterinarians for fighting injuries were FeLV antigen-positive, a prevalence considerably higher than in the clinically healthy cat population.[129] Although no breeds are predisposed to being infected with FeLV, infection is less commonly found in purebred cats, mainly because they are commonly kept indoors. In addition, awareness in the cat-breeder community leads to frequent testing. In older studies, young age also was considered to be a risk factor for FeLV infection, but this statement has to be reconsidered, too. In a study in the United States, in which 18,038 cats at 345 veterinary clinics and 145 animal shelters were tested, adults cats were more likely to be FeLV-infected than juveniles,[264] and in another study, the median age of FeLV-infected cats was not significantly lower than that of non-FeLV-infected cats,[127] at least in countries with good veterinary care. This is unexpected because the susceptibility of cats to FeLV is age-dependent,[194,201] but because of the increasing awareness, more cats are tested for FeLV, FeLV infection is recognized earlier, and medical care is provided during the initial stage of disease. In addition, awareness among cat breeders and animal shelters has led to routine testing of new pets entering the household or shelter. Moreover, euthanasia of infected asymptomatic cats is less common.

As demonstrated earlier, there is a significant decrease in prevalence of infection in many countries. However, with few exceptions, FeLV prevalence studies are uniquely based on detection of FeLV p27 antigen in blood using ELISA or similar immunochromatographic assays. But the pathogenesis of FeLV infection is complex, and free antigen can only be detected in the blood of cats with productive viremia, because those with regressive infections only harbor provirus in their bone marrow cells after overcoming antigenemia.[377] Thus, antigen testing may underestimate the true prevalence of infection. In a study in Switzerland it was shown that in addition to 7% of cats with both viral p27 antigen and provirus in blood, 10% of cats had negative results for p27 antigen and positive results for proviral DNA in blood.[189] This result is surprisingly high and raises the question whether the same situation occurs in other countries.

*References 15, 20, 89, 97, 126, 264, 318, 462.

Transmission

FeLV is contagious and spreads through close contact between virus-shedding cats and susceptible cats. Transmission of FeLV occurs primarily via saliva, where the concentration of virus is higher than in blood. Viremic cats constantly shed millions of virus particles in saliva, and shedding through saliva occurs relatively consistently in FeLV-viremic cats.[131,132] The concentration of virus in saliva and blood of healthy viremic cats is as high as it is in those with signs of illness. FeLV is passed effectively horizontally among communal cats that have prolonged close contact. Fighting and biting behavior,[127,129] as well as social behavior, such as sharing food and water dishes, mutual grooming, and using common litter areas, are the most effective means of transmission. Although the virus may enter many tissues, body fluids, and secretions, it is less likely to spread via urine and feces, and urine and feces were not considered an important source of infection. However, it was shown that antigenemic cats shed FeLV RNA and DNA in feces and urine, and infectious virus was isolated from feces and urine.[50,130] It was even shown that naïve cats exposed to virus-containing feces developed anti-FeLV antibodies, showing that infection through feces without direct cat-to-cat contact took place, but these cats remained negative for FeLV antigen and provirus in blood. These results suggest that fecal shedding of FeLV may play a role in transmission, but is probably of minor importance under natural circumstances. Nevertheless, sharing of litter pans by susceptible and viremic cats could increase the environmental infectious pressure.[130] Fleas have been considered a potential source of transmission because FeLV RNA has been detected in fleas and their feces,[448,449] but flea transmission does not seem to play a major role in nature. Iatrogenic transmission can occur via contaminated needles, instruments, fomites, or blood transfusions.[279]

The viral envelope is lipid-soluble and susceptible to disinfectants, soaps, heating, and drying. FeLV is readily inactivated in the environment within minutes. Therefore, close contact among cats is usually required for spread of infection, and indirect transmission (e.g., via feces-contaminated humans) is hardly possible. Single cats kept strictly indoors are not at risk for acquiring infection. It is only because of latency (in regressively infected cats) and potential reactivation that viremia is occasionally detected in middle-aged to old cats that have lived alone indoors since they were adopted as kittens. Because of the viral lability, a waiting period is not needed before introducing a new cat into a household after removal of an infected cat. FeLV is not a hazard in a veterinary hospital or boarding kennel as long as cats are housed in separate cages and routine cage disinfection and hand washing are performed between handling cats. FeLV is maintained in nature because infected cats may live and shed virus for many years.

Vertical transmission from mother to kittens occurs commonly in FeLV-viremic cats. Neonatal kittens can be infected transplacentally or when the queen licks and nurses them. Transmission also can occur in queens that are regressively infected (and therefore, have a negative result on routine tests) because latent infection may be reactivated during pregnancy. In addition, isolated FeLV transmission via milk to offspring, from queens with antigen-negative test results, has been described. If in utero infection occurs, reproductive failure in the form of fetal resorption, abortion, and neonatal death is common, although up to 20% of vertically infected kittens may survive the neonatal period to become persistently infected adults.[257] It is possible to observe that newborn kittens from infected queens have negative FeLV antigen test results at the time of birth but may have positive test results over the following weeks to months once the virus starts replicating. Thus, if the queen or any kitten in her litter is infected, the entire family should be treated as if infected and should be isolated from uninfected cats.

Susceptibility to becoming persistently FeLV viremic is highest in young kittens. Studies in a household with many FeLV-infected cats showed that 7 of 10 kittens placed there at 3 months of age became viremic within 5 months, whereas only 3 of 17 adults in the same household became viremic over 7 years.[58,59] Experimental infection is difficult if not impossible in healthy adult cats. Depending on the FeLV strains used, experimental infection can even be difficult to achieve in kittens older than 16 weeks of age.[194] Age resistance to FeLV also exists in nature. Prevalence of anti-FeLV antibodies increases steadily over time, indicating an increased exposure to the virus throughout life, and although exposure to FeLV accumulates with age, susceptibility to develop persistent viremia after infection simultaneously decreases. The described age resistance is independent of immunity from previous contact or vaccination. An explanation for the age resistance is that the number of cellular receptors necessary for FeLV-A to enter target cells seems to decrease in older cats, and thus, establishment of infection becomes more difficult. Age resistance also may be related to maturation of macrophage function.[191] However, age-related resistance is not absolute and depends on the infection pressure. Risk of developing persistent viremia increases in kittens but also to a certain extent in adult cats when they are housed together with FeLV-shedding cats. This is shown by the increased rate of viremic cats in households with endemic FeLV infection and by natural exposure studies in which a certain percentage of cats becomes FeLV-positive over years when they are housed together with infected cats. However, the risk of an adult cat becoming persistently viremic after one short contact with a FeLV-shedding cat is certainly very low and probably lower than the risk of developing vaccine-associated sarcomas after FeLV vaccination. Therefore, use of FeLV vaccination should be considered carefully in adult cats.

The cellular receptors of FeLV are not fully identified despite intensive ongoing research. FeLV subgroups use different receptors,[40,308,364,402] and strain-dependent differences seem to exist. A binding receptor for FeLV-A has been detected that seems to be identical with the feline thiamine transport protein 1 (THTR1) receptor.[308] FeLV-C uses the host receptor known as FLVCR1, but binding of FeLV-C to FLVCR1 seems to involve interaction of two receptor-binding domains (including the carboxy terminal C domain) with the host receptor FLVCR1.[364] FeLV-B uses a cellular protein (phosphate transporter 1, Pit-1) as receptor.[40,402] FeLV-T also can use Pit-1 as a receptor but the host ranges of FeLV-B and FeLV-T are not exactly the same, suggesting a different Pit-1 use at the postbinding level.[402] FeLV-T cannot infect cells unless a classic multiple membrane-spanning receptor molecule and a second co-receptor or entry factor are present. This cellular protein can function as either a transmembrane protein or a soluble component to facilitate infection.[10]

PATHOGENESIS

The outcome of FeLV infection is very different in each cat. Although outcome mainly depends on immune status and age of the cat, it is also affected by pathogenicity of the virus, infection pressure, and virus concentration.[161] Outcome of FeLV infection also reflects genetic variation both in the virus and the naturally outbreeding host population. Mutational changes identified in FeLV strains were shown not to alter receptor usage, but to significantly increase the efficiency of receptor binding. Longitudinal studies of infected animals showed that certain mutations resulted in a significantly more rapid disease onset, whereas other substitutions in certain genes changed the disease outcome entirely, suggesting that the distinctive LTR and surface unit (SU) genes mediate a rapid pathogenesis with distinctive clinical features and oncogenic mechanisms.[257]

Stages of Feline Leukemia Virus Infection

Discussions of FeLV infection, which has different courses, outcomes, and classifications (Figs. 11-4 and 11-5 and Table 11-3) are controversial. Diagnostic tools, including very sensitive polymerase chain reaction (PCR) methods, have provided new data that question the traditional understanding of FeLV pathogenesis. Previously, most FeLV pathogenesis studies were conducted assaying parameters such as virus isolation and antigen detection. Accordingly, infection was characterized by undetectable, transient, or persistent viremia. Using real-time PCR, the spectrum of host response categories to FeLV infection was refined by investigating proviral DNA and viral RNA loads. Cats believed to be immune to FeLV infection were found to have positive provirus test results. FeLV provirus was found to persist for years; recurrence of viremia and disease development was observed in some cats. Thus, cats with negative antigen and positive provirus test results are FeLV carriers and, after reactivation, may act as an infection source. However, integrated viral DNA may also be essential for solid protection and long-lasting maintenance of protective immunity.[187] Therefore, the potential courses of FeLV infection have

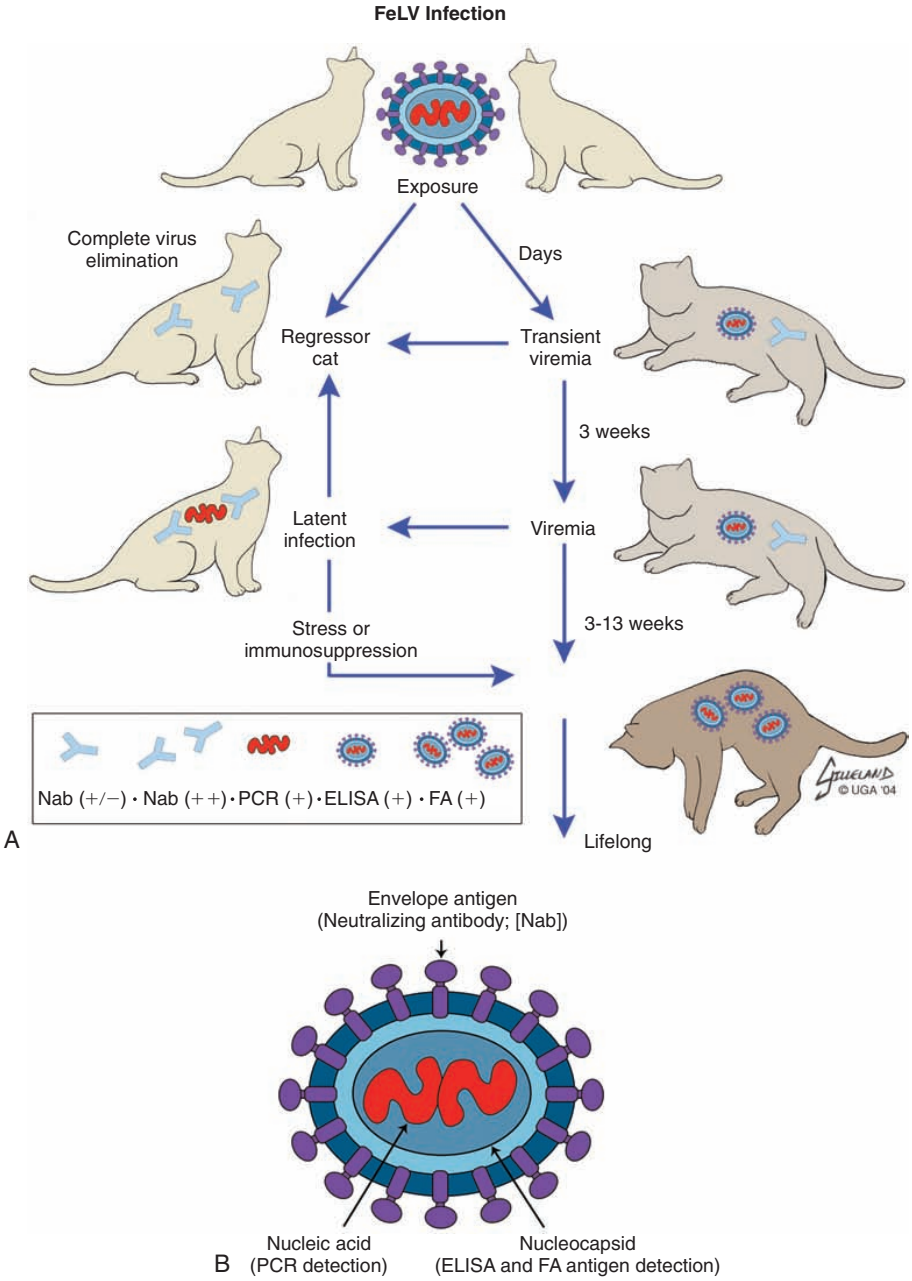

FIG. 11-4 ***A,*** Time course of FeLV infection. ***B,*** Components of FeLV from part A. *ELISA*, Enzyme-linked immunosorbent assay; *FA*, fluorescent antibody; *FeLV*, feline leukemia virus; *PCR*, polymerase chain reaction. (Art by Brad Gilleland © 2004, University of Georgia Research Foundation Inc.)

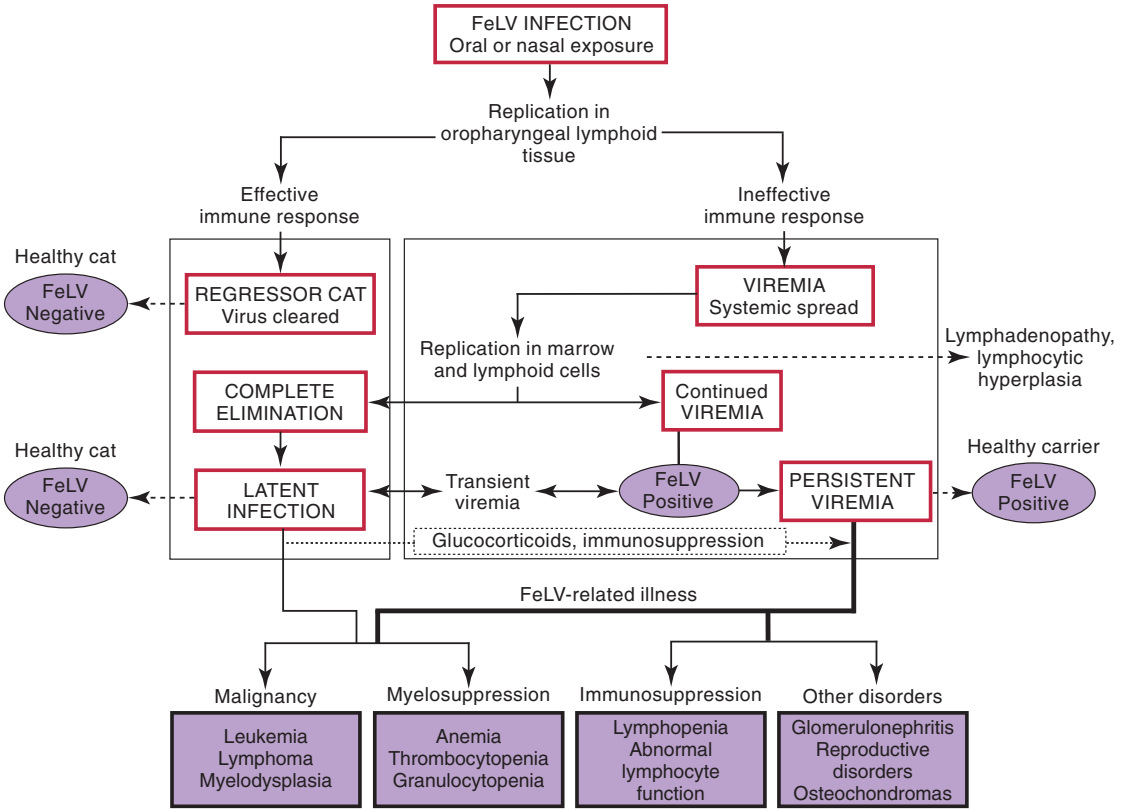

FIG. 11-5 Interactions of feline leukemia virus (FeLV) with host cells and immune system leading to various clinical problems in cats with ineffective immune responses.

TABLE 11-3

Characteristics of Stages of Feline Leukemia Virus Infection

Outcome of FeLV Infection	FeLV p27 Antigen in Blood	Virus Blood Culture	Viral RNA in Blood	Viral DNA in Blood	Viral Tissue Culture	Viral Shedding	FeLV-Associated Disease
Progressive	Positive	Positive	Positive	Positive	Positive	Positive	Likely
Regressive	Negative	Negative	Negative	Positive	Negative	Negative	Unlikely
Abortive	Negative	Negative	Negative	Negative	Negative	Negative	Unlikely
Focal	Negative	Negative	Not tested	Not tested	Positive	Variable	Unlikely

Progressive = persistent viremia; Regressive = transient viremia followed by latent infection; Abortive = complete elimination.
From Ref. 260.

been reclassified, and the stages of FeLV infection are described as (1) abortive infection (comparable to the former "regressor cats"), (2) regressive infection (comparable to the former "transient viremia" followed by "latent infection"), (3) progressive infection (comparable to the former "persistent viremia"), and (4) focal or atypical infection (see Table 11-3).[186,187,436]

In the past, approximately one-third of cats were believed to become persistently viremic and up to two thirds of cats eventually clear the infection.[191] Newer research suggests that most cats remain infected for life after exposure but may revert to an aviremic state (regressive infection) in which no antigen or culturable virus is present in the blood but in which FeLV proviral DNA can be detected in the blood by sensitive PCR methods.[189,343,436] The clinical relevance of cats with antigen-negative and provirus-positive results is not yet clear. The provirus is integrated into the cat's genome, so it is unlikely to be cleared over time.[49] Although these cats are unlikely to shed infectious virus in saliva, proviral DNA might be infectious via blood transfusion.[52] The continuous presence of provirus might explain the long persistence of virus-neutralizing antibodies in "recovered" (recovered from viremia, but not from latent infection) cats. Before the development of PCR, a status of "latent infection" was described in which the absence of antigenemia was accompanied by persistence of culturable virus in bone marrow or other tissues but not in blood.* The "latent infection" is now considered a phase through which cats pass during regressive infection.[33] FeLV provirus and plasma viral RNA are usually detectable by PCR within 1 week of FeLV exposure,

*References 186, 290, 334, 340, 351, 377.

even if FeLV antigen is not. All cats with progressive and regressive infection seem to undergo this phase and to develop similar proviral and plasma viral RNA loads in the peripheral blood during early infection.[187] After FeLV exposure, FeLV infection has four possible outcomes, described next (see Table 11-3).

Abortive Infection

After initial infection, which most commonly occurs via oronasal routes, virus replicates in the local lymphoid tissue in the oropharyngeal area. In some immunocompetent cats, viral replication may be stopped by an effective humoral and cell-mediated immune (CMI) response; these cats never become viremic. This abortive exposure has been observed infrequently after experimental FeLV inoculation and is characterized by negative test results for culturable virus, antigen, viral RNA, and proviral DNA.[436] These cats were formerly called "regressor cats." They have high levels of neutralizing antibody, but neither FeLV antigen nor viral RNA or proviral RNA can be detected in the blood at any stage. In these cats, virus never spreads systemically, and infection usually remains undetected. Abortive infection likely is caused by low-dose exposure to FeLV, as shown in an experimental study in which, after exposure to low doses of FeLV, cats only developed antibodies as the sole parameter of infection.[292] It is currently unknown how often this situation truly occurs in nature, because newer studies using very sensitive PCR methods have found that in many of the formerly considered "regressor cats," virus actually can be retrieved later on, and it appears likely that no cat or only very few can completely clear FeLV infection from all cells. This might explain why virus-neutralizing antibodies persist in recovered cats for many years (or even lifelong) in the absence of overt infection or exposure to viremic cats. If this is the case, the risk of such persistence leading to potential reexcretion of virus or the development of FeLV-associated disease must be extremely low, because recovered cats appear to have the same life expectancy as cats that have never been exposed to FeLV.[279] This explains why the majority of cats in a population show evidence of exposure by the presence of anti-FeLV antibodies after contact with FeLV, but only a small proportion actually become viremic. These cats build a very effective immunity and are protected against new viral challenges, probably for several years if not lifelong. Protective immunity is partly humoral and partly cellular, and antibody production is not necessarily required for protection; about 2% are effectively protected without detectable antibodies.

Regressive Infection

Regressive infection is accompanied by an effective immune response, and virus replication and viremia are contained before or shortly after the time of bone marrow infection. After initial infection, replicating FeLV spreads systemically within mononuclear cells (lymphocytes and monocytes). During this first viremic episode, free FeLV-p27 antigen is detectable, and cats have positive results on tests that detect free antigen in plasma (e.g., ELISA) and can shed the virus during that period. The initial viremia may be characterized by malaise, fever, or lymphadenomegaly resulting from lymphocytic hyperplasia. The virus spreads to target tissue including thymus, spleen, lymph nodes, and salivary glands. In cats with regressive infection, this viremia is terminated within weeks or months (formerly called "transient viremia"). In most cats, the viremia lasts only 3 to 6 weeks (with a maximum of 16 weeks). During this time, cats shed virus and are infectious. Many cats are able to clear viremia very early before bone marrow becomes infected. It was thought that these cats not only terminate the viremia, but also completely eliminate the virus from the body. However, studies question the fact that virus can be completely cleared and that virus may be found in these cats at a later time. These cats also develop a very effective immunity and are protected against new exposures to virus. They have a low risk of developing FeLV-associated diseases, although FeLV is integrated into their genome (and thus, FeLV can be detected by PCR). After virus replication is contained, viral shedding does not occur.[109,110,282]

In some cats, viremia may persist longer than 3 weeks. After this time period, bone marrow cells may become infected, and affected hematopoietic precursor cells produce infected granulocytes and platelets that circulate in the body. In this circumstance, a high level of viremia develops, and lymphoid organs and salivary glands become infected with up to 1×10^6 viruses/mL of saliva. From this time point on, viral antigen is also detectable intracellularly in platelets and granulocytes by tests such as direct fluorescent antibody (FA) assays that can only detect large quantities of intracellular antigen. In contrast to antigen tests (e.g., ELISAs) that can detect lower quantities of free p27 antigen and become positive during the first viremia, direct FA test results become positive later and only after infection is established in bone marrow. This explains discordant ELISA-positive and direct FA-negative results. Even if bone marrow becomes infected, a certain percentage of cats can clear viremia (and therefore develop regressive infection); however, the longer the viremia lasts, the less likely it is that these cats will clear their infection. Once bone marrow cells develop an established infection (after 3 weeks of viremia), cats cannot eliminate the virus from the body and from the bone marrow even if they terminate viremia because the information for virus replication (its proviral DNA) is present in bone marrow stem cells. This stage has been called "latent infection" (now considered a stage of regressive infection). Although proviral DNA remains, no virus is actively produced, and cats with regressive infection have negative results from routine tests (ELISA and FA) that detect FeLV antigen. Regressive infection can only be diagnosed by in vitro culture of bone marrow samples or using PCR to detect provirus. Growth can be facilitated by adding glucocorticoids to the cell culture. Productive viral infection can be reactivated in vivo, spontaneously or in response to immune suppression, and latently infected cats can become viremic and show positive results again in antigen tests. This usually occurs after stress and can be experimentally induced in cats by administration of high doses of glucocorticoids.[377]

Regressive infections may reactivate in pregnancy as a result of immunosuppression from endogenous progesterone, which also may explain the reemergence of FeLV infection in kittens. Mammary glands of regressively infected queens may begin to produce infectious viral particles during the induction of lactation.[334]

Regressive infection and the latent state are unique features in FeLV infection. The molecular basis of latency is the integration of a copy of the viral genome (provirus) into cellular chromosomal DNA. During the replication cycle, the enzyme RT produces a DNA copy using the viral RNA as a template. The copy is integrated into the cellular chromosomal DNA and maintained as a provirus for the life span of the cell. During cell division, proviral DNA is replicated and the information given to the daughter cells. Thus, complete cell lineages may contain FeLV proviral DNA. However, the proviral DNA is not translated into proteins, and no infectious virus particles are produced. Therefore, regressively latently infected cats do not shed FeLV and are not infectious to other cats. Although latency is a sequel to FeLV infection, the majority of cats completely eliminate the viral genes from their cells by 9 to 16 months after infection, and all but 10% have done so after 30 months.[334] Virus can remain integrated in a small number of cells for a long time, while being kept in check by a partial immune response. As antibody concentration increases, virus production decreases. No harmful virus is produced during regressive infections, and clinical signs (with few exceptions such as neoplasia or myelosuppressive syndromes) do not occur. In a study in Switzerland, where 7% of cats had both positive p27 antigen and

positive proviral test results, 10% of the cat population had negative p27 antigen results and positive proviral test results in blood, which indicates latent infection.[189]

Regressive infection can be reactivated because the genetic information for producing complete viral particles is present and can potentially be reinduced when antibody production decreases (e.g., after immune suppression). Reactivation is more likely the earlier the stress factor occurs after the viremic phase. In the first weeks after viremia, viral replication can be experimentally reactivated in most cats. As the time passes, regressive infections become more difficult to reactivate, even with high doses of glucocorticoids. Although possible by 1 year after infection, reactivation is considered unlikely and is very difficult after 2 years. This may be explained by genetic code-reading mistakes that may occur if the information is frequently reproduced in these fast-dividing cells. Thus, information to produce infectious viral particles gets lost, and reactivation becomes more and more unlikely over time. The proportion of experimentally infected cats that had regressive FeLV infections in their bone marrow decreased with time after disappearance of viremia.[340] In the first 3 months after recovery from viremia, integrated virus could be isolated from the bone marrow of approximately 50% of experimentally infected cats. A pronounced decrease in the incidence of regressive infections occurred by 190 days after the viremia.[334,340] More than 1 year later, only 5 of 19 previously challenge-exposed cats that had negative FeLV ELISA test results still had FeLV detectable in several tissues (e.g., bone marrow, spleen, lymph node, small intestine).[174] At 3 years postviremia, only about 8% of cats still harbored latent infections in bone marrow, myelomonocytic cells, and stromal fibroblast cells.[189,290,334,340,377] Regressive infection is probably a stage in the elimination process of the virus.

Most regressive infections are not clinically significant because viral reactivation is unusual under natural circumstances. As long as the infection remains confined, the cats are not contagious. However, viral latency explains relapsing viremias, protracted incubation periods, and persistent high titers of antibodies. A question always arises regarding whether regressive FeLV infection can be responsible for clinical signs. However, for the majority of pathogenic mechanisms by which FeLV causes clinical signs, active virus replication is necessary; but this is not the case in regressive FeLV infections, in which the virus is harbored in a "dormant" and nonproductive form. Regressive, as compared to productive, FeLV infection has been found to occur most commonly in older cats that originated from animal shelters and, rather than lymphoma, was more commonly associated with anemia, panleukopenia and purulent inflammatory processes.[419a] Regressive infections help to explain how myelosuppression or hematopoietic malignancy could be FeLV-related in cats with negative FeLV antigen test results. In one study, 2 of 37 cats (5%) with nonregenerative cytopenias and negative FeLV antigen test had positive results with bone marrow PCR, suggesting that regressive FeLV infection can cause myelosuppression.[419] Some studies also detected FeLV provirus in lymphomas of cats that had negative results on blood testing for FeLV antigen.[122,204] FeLV provirus can be inserted at many different sites in the host's genome, carrying potent regulatory signals. In the development of myelosuppressive disorders or tumors, integrated FeLV provirus may interrupt or inactivate cellular genes in the infected cells, or regulatory features of viral DNA may alter expression of neighboring genes. In addition, because bone marrow microenvironment cells (e.g., myelomonocytic progenitor cells and stromal fibroblasts) provide a reservoir for regressive FeLV infections, it seems possible that the integrated provirus may alter cellular functions and contribute to the pathogenesis of myelosuppressive disorders. Finally, FeLV not only contributes its genes to the host, it also has been shown to appropriate cellular genes. Several such transducted genes that are also present in regressively infected cells have been implicated in viral oncogenesis.[365,373,395] In one SPF cat, experimentally infected with FIV and FeLV, regressive infection occurred and the cat became aviremic for 8.5 years. A genetically altered FeLV variant of this virus reappeared in the blood, in conjunction with the development of multicentric lymphosarcoma.[175a]

Progressive Infection

In cats with progressive FeLV infection, virus is not contained early in the infection, and extensive replication occurs, first in the lymphoid tissues and then in the bone marrow and in mucosal and glandular epithelial tissues in most infected cats.[376] Mucosal and glandular infection is associated with excretion of infectious virus. In progressive infections, the immune response is not strong enough; thus, viremia persists longer than 16 weeks, and cats remain persistently viremic and infectious to other cats for the remainder of their lives. This condition was called "persistent viremia" and is now designated as progressive infection. Progressive infection is characterized by insufficient FeLV-specific immunity. Progressively infected cats have low levels of detectable neutralizing antibody, and virus persistently replicates in bone marrow, spleen, lymph nodes, and salivary glands. These cats develop FeLV-associated diseases, and most of them die of an FeLV-related disease within 3 years.

The risk for the development of a fatal progressive infection primarily depends on immune status and age of the cat, but also on the infection pressure. Young and immunosuppressed cats are at higher risk for developing progressive infection. In a cat with a first-time single contact with an FeLV-shedding cat, the risk of developing progressive infection averages only 3%. However, if an FeLV-shedding cat is introduced into a naïve group of cats, and the cats are housed together for an extended period, the risk for a cat to develop progressive infection increases to an average of 30%.[19]

Regressive and progressive infections can be distinguished by repeated testing for viral antigen in peripheral blood.[436] Many infected cats initially develop positive antigen test results within 2 to 3 weeks after virus exposure. In case of regressive infection a cat develops a negative viral antigen test result 2 to 8 weeks later or, in rare cases, even after months. Both progressive and regressive infections are almost always accompanied by persistent FeLV proviral DNA in blood. However, FeLV proviral and viral RNA loads in leukocyte subsets, as analyzed by quantitative PCR, indicate that FeLV progressive infection is associated with secondary viremia of bone marrow origin, whereas regressive cats sustain only a nonproductive viral infection in low numbers of lymphocytes.[47] During acute infection, blood proviral and viral RNA loads of cats with progressive and regressive infections are not significantly different. Only subsequently, the infection outcome is associated with different FeLV loads, and it is not the overall loads but rather those of specific leukocyte subsets that may influence the infection outcome.[343]

Focal or Atypical Infection

Focal infections or atypical infections have been reported in early studies in up to 10% of infected cats under experimental conditions. Focal or atypical infections may also rarely be observed in natural infections, consisting of persistent atypical local viral replication (e.g., in mammary glands, bladder, and eyes).[191] They also occur in cats with FeLV infection restricted to certain tissues, such as the spleen, lymph nodes, small intestine, or mammary glands.[175,335] This can lead to intermittent or low-grade production of p27 antigen. Therefore, these cats may have weakly positive or discordant results in antigen tests, or positive and negative results may alternate. Queens with atypical infection of their mammary glands may transmit the virus to their kittens via milk in the phase of negative antigen test results.

Immunity

Experimentally, susceptible kittens can be protected from FeLV infection after passive immunization with sera containing high antibody concentrations against FeLV.[196] However, once persistent viremia has become established, treatment with virus-neutralizing monoclonal antibodies (MABs) to FeLV is ineffective.[452]

Most cats that overcome FeLV viremia exhibit high antibody titers against the virus.[283,381] Antibodies are directed against all components of the virus.[283] In most but not all cats that overcame viremia, virus-neutralizing antibodies can be detected.[109] Because not all immune cats develop high antibody levels, it was concluded that cytotoxic T lymphocytes (CTLs) are also important in FeLV immunity.[283] CTLs specific for FeLV appear before virus-neutralizing antibodies and the virus load in FeLV-progressively infected cats could be lowered, after passive transfer of FeLV specific CTLs stimulated in vitro, consistent with an important role for CTLs in FeLV immunity.[109]

CLINICAL FINDINGS

FeLV can cause variable clinical signs. The prevalence of hematopoietic malignancy, myelosuppression, and infectious diseases is higher in FeLV-infected multicat households than in the general population. The death rate of progressively infected cats in multicat households has been considered approximately 50% in 2 years and 80% in 3 years.[62,257] However, survival rates for progressively infected cats kept indoors in single-cat households with good veterinary care today are significantly higher. In contrast, FeLV infection has the greatest impact on mortality in closed households with endemic feline coronavirus, FeLV, FIV, or all of these infections.[3] A large study in the United States compared the survival of more than 1000 FeLV-infected cats to more than 8000 age- and sex-matched uninfected control cats[263] and found that in FeLV-infected cats, median survival was 2.4 years compared to 6 years for control cats.

Although the virus was named after the contagious malignancy that first garnered its attention, most infected cats are presented to the veterinarian not for tumors but for anemia or immunosuppression. Of 8642 FeLV-infected cats examined at North American veterinary teaching hospitals, various co-infections (including FIP, FIV infection, upper respiratory infection, hemotropic mycoplasmosis, and stomatitis) were the most frequent findings (15%), followed by anemia (11%), lymphoma (6%), leukopenia or thrombocytopenia (5%), and leukemia or myeloproliferative disease (4%).[59]

The exact mechanisms for the different clinical responses of progressively infected cats are poorly understood. It is clear that the clinical course is determined by a combination of viral and host factors. Some of these differences can be traced to properties of the virus itself, such as the subgroup that determines differences in the clinical picture (e.g., FeLV-B is primarily associated with tumors, FeLV-C is primarily associated with nonregenerative anemia). A study tried to define dominant host immune effector mechanisms responsible for the outcome of infection by using longitudinal changes in FeLV-specific CTLs. As mentioned previously, high levels of circulating FeLV-specific effector CTLs appear before virus-neutralizing antibodies in cats that have recovered from exposure to FeLV. In contrast, progressive infection with persistent viremia has been associated with a silencing of virus-specific humoral and CMI host effector mechanisms.[109] Probably the most important host factor that determines the clinical outcome of cats infected with FeLV is the age of the cat at the time of infection.[194] Neonatal kittens develop marked thymic atrophy after infection ("fading kitten syndrome"), resulting in severe immunosuppression, wasting, and early death. As cats mature, they acquire a progressive resistance. When older cats become infected, they tend to have abortive or regressive infections or, if developing progressive infection, at least milder signs and a more protracted period of apparent good health.[257] Clinical signs that are associated with FeLV infection can be classified as tumors induced by FeLV, hematologic disorders, immunosuppression, immune-mediated diseases, and other syndromes (including reproductive disorders, fading kitten syndrome, and neuropathy).

Tumors

FeLV causes various tumors in cats, most commonly malignant lymphoma and leukemia and less commonly other hematopoietic tumors. Lymphomas also occur in the absence of detectable FeLV.[451] In addition, other tumors, including osteochondromas, olfactory neuroblastoma, and cutaneous horns, have been described in FeLV-infected cats.

The mechanism by which FeLV causes malignancy may be explained by insertion of the FeLV genome into the cellular genome near a cellular oncogene (most commonly *myc*), resulting in activation and overexpression of that gene. These effects lead to uncontrolled proliferation of that cell (clone). A malignancy results in absence of an appropriate immune response. FeLV-A may also incorporate the oncogene to form a recombinant virus (e.g., FeLV-B, FeSV) containing cellular oncogene sequences that are then rearranged and activated. When they enter a cell, these recombinant viruses are oncogenic. In a study of 119 cats with lymphomas, transduction or insertion of the *myc* locus had occurred in 38 cats (32%).[439] Thus, FeLV-induced neoplasms are caused, at least in part, by somatically acquired insertional mutagenesis in which the integrated provirus may activate a proto-oncogene or disrupt a tumor suppressor gene. The U3-LTR region of FeLV transactivates cancer-related signaling pathways through production of a noncoding 104 base RNA transcript that activates NFκB.[112] Common integration sites for FeLV associated with lymphoma development have been identified in six loci: *c-myc*, *flvi-1*, *flvi-2* (contains *bmi-1*), *fit-1*, *pim-1*, and *flit-1*. Oncogenic association of the loci includes that *c-myc* is known as a proto-oncogene; *bmi-1* and *pim-1* have been recognized as *myc*-collaborators; *fit-1* appears to be closely linked to *myb*; and *flit-1* insertion was shown to be associated with overexpression of cellular genes, such as activin-A receptor type II-like 1 (*ACVRL1*).[120] *Flit-1* seems to have an important role in the development of thymic lymphomas and appears to represent a novel FeLV proviral common integration domain that may influence lymphomagenesis as insertional mutagenesis. Among 35 FeLV-related tumors, 5 of 25 thymic lymphomas demonstrated proviral insertion within the *flit-1* locus, whereas none of 4 alimentary and 5 multicentric lymphomas and 1 T-lymphoid leukemia examined had rearrangement in this region. Expression of *ACVRL1* messenger RNA (mRNA) was detected in the 2 thymic lymphomas with *flit-1* rearrangement, whereas normal thymuses and 7 lymphoid tumors without *flit-1* rearrangement had no detectable *ACVRL1* mRNA expression.[119]

Feline oncornavirus cell membrane antigen (FOCMA), an antigen present on the surface of transformed cells, was detected in 1973 but remains a subject of discussion and confusion among researchers. Its value as clinical tool (either diagnostic or preventative) is certainly limited. FOCMA was first detected on the surface of cultured lymphoma cells incubated with serum of cats that did not develop tumors, although they were infected with FeSV, a recombinant of FeLV with an oncogenic potential.[102,407] FOCMA can be found on the surface of feline lymphoma cells and FeSV-induced fibrosarcomas but not on the surface of normal feline lymphocytes.[154,447] FOCMA was first considered to be a cellular antigen that is expressed after FeLV infection or tumor transformation.[100,154,407] It has also been proposed that FOCMA is a viral antigen of FeLV-C.[447] However, in other studies it

was shown that FOCMA and FeLV-C-gp70 are similar but not completely homologous.[408] Some authors believed that development of large amounts of antibodies against FOCMA could protect against the development of FeLV-induced lymphomas by complement-dependent lysis of tumor cells.[63,98,133] Evidence for this was provided when experimentally FeLV-infected kittens did not develop neoplasia if they produced or passively received sufficient amounts of antibodies against FOCMA.[99,102] Many cats with FeLV in cluster households have antibodies against FOCMA. Those with the highest titers are most likely to remain free of malignancies. However, some cats that were initially viremic with a high FOCMA antibody titer developed lymphoma or leukemia months or years later after the titer declined.[62] Opinions about identity and importance of FOCMA are still diverse. FOCMA can be considered a nonhomogenous group of viral antigens that may, although not always, be present on the surface of FeLV-infected cells. At the least, FOCMA antibodies indicate exposure to FeLV but may not mean more than this. Alternatively, FOCMA antibodies may provide a protective mechanism against tumor development.

Lymphoma and Leukemia

In the 1960s, studies found that the most common primary feline malignancies are hematopoietic tumors, of which about 90% are lymphomas. Lymphomas and leukemias account for about 30% of all feline tumors, which is the highest proportion recorded in any animal species.[66,87,88,145,146] The estimated incidence of feline lymphoma and leukemia in the 1960s was 200 cases per 100,000 cats per year.[62] Feline lymphomas are most commonly high grade with an immunoblastic or a lymphoblastic morphology, but they may be mixed lymphoblastic and lymphocytic or occasionally low-grade lymphocytic.[444,445]

The association between FeLV and lymphomas has been clearly established in several ways. First, these malignancies could be induced in kittens by experimental FeLV infection.[150,215,368] Second, cats naturally infected with FeLV had a higher risk of developing lymphoma than uninfected cats.[98,150] Third, most cats with lymphoma had positive FeLV results on tests that detected infectious virus or FeLV antigens. Previously, up to 80% of feline lymphomas and leukemias were reported to be FeLV related*; however, this is no longer considered to be the case. Since the 1980s a dramatic reduction in the prevalence of viremia has been noted in cats with lymphoma.[167,299,314] The decrease in prevalence of FeLV infection in cats with lymphoma or leukemia also indicates a shift in tumor causation. Whereas 59% of all cats with lymphoma or leukemia had positive FeLV antigen tests in one German study from 1980 to 1995, only 20% of the cats had positive FeLV antigen tests in the years 1996 to 1999 in the same institution.[167] In 1975 a survey of 74 Boston-area cats with lymphoma or leukemia showed that 70% of cats had positive FeLV antigen test results, but only three cats had the alimentary form.[58] Between 1988 and 1994, 72% of all feline lymphomas treated at the Animal Medical Center in New York were of the alimentary form, and only 8% of affected cats had positive FeLV antigen test results.[62] In a study in the Netherlands, only 4 of 71 cats with lymphoma had positive FeLV antigen test results, although 22 of these cats had mediastinal lymphoma, which was previously highly associated with FeLV infection.[430] A greater prevalence of lymphoma in older cats has been observed. One major reason for the decreasing association of FeLV with lymphoma seems to be the decreased prevalence of FeLV infection in the overall cat population as a result of FeLV vaccination as well as testing and elimination programs.

Overall, the proportion of cats with lymphomas that have negative test results for the FeLV antigen (versus cats with lymphomas that have positive results) has increased significantly during the past 20 years. However, prevalence of lymphomas caused by FeLV may be higher than indicated by conventional antigen testing of blood. Cats from FeLV-cluster households had a 40-fold higher rate of development of FeLV-negative lymphoma than did those from the general population. FeLV-negative lymphomas have also occurred in laboratory cats known to have been infected previously with FeLV.[373] FeLV proviral DNA was detected in lymphomas of older cats that had positive test results for FeLV antigen, also suggesting that the virus may be associated with a larger proportion of lymphomas than previously thought. PCR detected proviral DNA in formalin-fixed, paraffin-embedded tumor tissue in 7 of 11 cats with negative FeLV antigen test results with lymphoma.[204] However, other groups found evidence of provirus in only 1 of 22[395] and in none of 50 FeLV antigen test result–negative lymphomas.[167] FeLV antigen test result–negative lymphomas induced by FeLV can be explained in various ways. First, regressive FeLV infection without viremia may be responsible for the tumor development. Second, FeLV could be responsible for the development of the tumor, inducing a malignant cell clone, but not be persistently integrated into the genome of the neoplastic cell and, therefore, be eliminated while the tumor grows to a detectable size. Third, FeLV infection could be present in other cells (and not detectable) and induce oncogenesis via mechanisms such as cytokine release or chronic immune stimulation.

The FeLV status of cats with lymphomas varies depending on the types and locations of tumors. FeLV-associated lymphomas are mainly of a T-cell origin; FeLV test result–negative lymphomas are mainly of a B-cell origin.[113,146,154,323] A potential reason may be that FeLV transforms mature T cells and immature or prothymocytes, null cells, and possibly monocytes. Transformation of mature B cells does not seem to occur, because feline lymphoma cell lines and primary tumors lack surface immunoglobulin expression.[379] The rare feline large granular lymphocyte lymphoma, a morphologically distinct variant of feline lymphoma with grave prognosis, does not seem to be commonly associated with FeLV. In a study of 45 cats with large granular lymphocyte lymphoma, none of the cats had positive results for FeLV antigen testing.[240] Similarly, low-grade lymphomas are usually not associated with FeLV; in a study of 41 low-grade lymphocytic lymphomas, none of the cats had positive results for FeLV antigen testing.[233] Lymphomas also can be classified according to their anatomic location, as mediastinal (thymic) lymphoma, alimentary (intestinal) lymphoma, multicentric lymphomas, extranodal (miscellaneous/atypical/solitary organ) lymphoma including renal, nasal, and ocular lymphoma, and leukemia.

Mediastinal lymphoma or *thymic lymphoma*, frequently associated with FeLV infection and seen mainly in cats younger than 3 years of age, was previously the most prevalent form of lymphoma in cats but is now seen less frequently. Of cats with mediastinal lymphoma, 80% to 90% have been reported as FeLV antigen test result-positive,[61] but this rate is also decreasing according to other studies,[430] and non-FeLV-associated mediastinal lymphoma even in young cats are observed.[390] In a study in Germany, none of 23 cats in the study were found to have positive FeLV antigen test results, although 4 of the cats had mediastinal lymphoma.[404] The tumor arises in the area of the thymus and eventually causes pleural effusion (Figs. 11-6 and 11-7). The fluid nucleated cell count is usually greater than 8000/μL; the majority are large, immature lymphocytes. The most common clinical sign is dyspnea, but occasionally regurgitation from pressure on the esophagus or Horner's syndrome from pressure on sympathetic nerves within the thorax is present.[62]

Alimentary lymphoma or *intestinal lymphoma* occurs primarily in older cats that have negative FeLV test results. Clinical signs of alimentary lymphoma include vomiting or diarrhea, but many cats have anorexia and weight loss only.[291] Tumors of the stomach and intestines

*References 63, 113, 118, 152, 157, 360, 396.

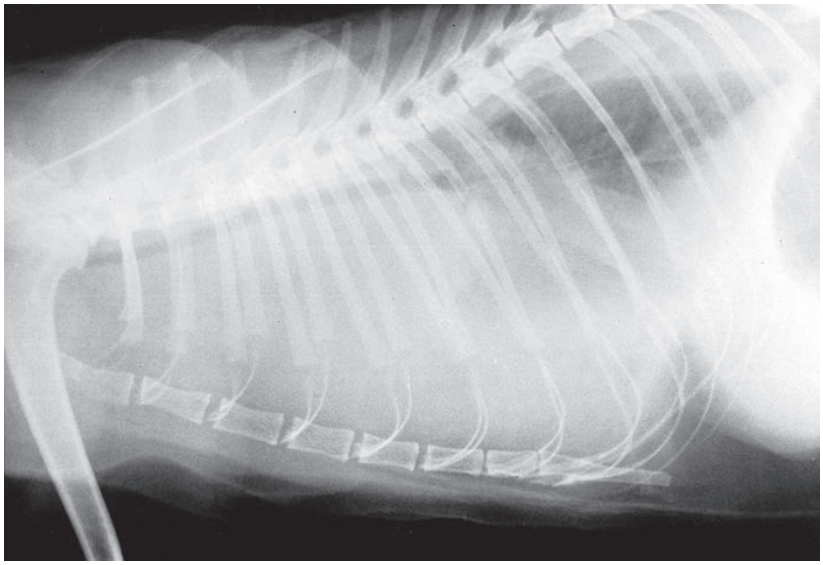

FIG. 11-6 Lateral thoracic radiograph of a cat with severe pleural effusion and mediastinal mass. The trachea is displaced dorsally, and the cardiac shadow is not shown.

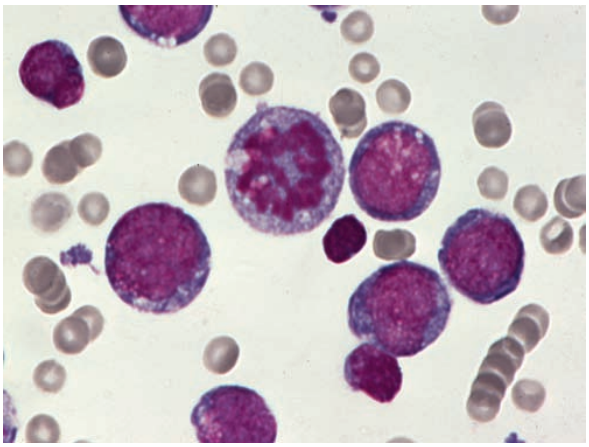

FIG. 11-7 Examination of thoracic fluid aspirated from a cat showing a pleomorphic lymphoid population composed of blasts, a mitotic figure, and a small lymphocyte. Diagnosis of lymphoma was made (Wright stain, ×1000). (Photograph by Ken Latimerl © 2004, University of Georgia Research Foundation Inc.)

may be focal or diffuse, and mesenteric lymph nodes are usually involved. Estimates of the prevalence of FeLV antigenemia in cats with alimentary lymphomas have ranged from 25% to 30%.[62] However, in another study, only 6% of cats with alimentary lymphomas had positive FeLV antigen test results, which is only about twice the FeLV prevalence of the general population in that area.[167] These data suggest that other stimuli (such as food antigens or components and/or inflammatory bowel disease) in the gastrointestinal (GI) tracts of older cats may be more important predisposing factors for tumor development.

Multicentric lymphoma is a tumor with major involvement of several sites. About half of cats with multicentric lymphoma have positive FeLV antigen test results. The bone marrow is involved in about 70% of these cats, even though complete blood cell counts (CBC) may be within reference limits.

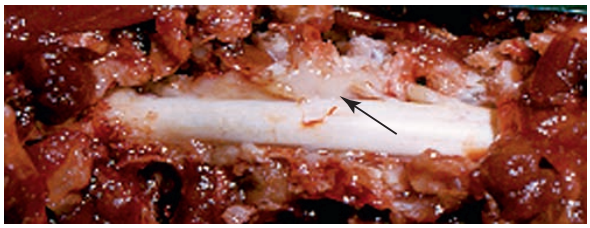

FIG. 11-8 Postmortem dissection of spinal canal reveals a cream-color gelatinous mass *(arrow)* in the epidural space. Histologic findings were diagnostic of lymphoma. (Photograph by Craig Greene © 2004, University of Georgia Research Foundation Inc.)

Extranodal lymphomas, *miscellaneous lymphomas* or *atypical lymphomas* or *solitary organ lymphomas,* unassociated with FeLV, have been observed with increased relative frequency over the past 20 years because of the decreased prevalence of FeLV and its associated lymphoreticular lymphomas. Extranodal lymphomas refer to disease confined to locations other than alimentary, mediastinal, nodal or multicentric sites and include renal, nasal/paranasal, central nervous system (CNS), ocular, laryngeal, and cutaneous lymphoma. As such, these atypical forms now make up approximately 20% of cases.[277] Of 149 cats with extranodal lymphoma, only 4 had positive FeLV antigen test results (3 nasal, 1 CNS lymphoma).[429] In another study, 5 of 51 cats with nasal lymphoma had positive FeLV antigen test results.[142] Renal lymphoma is sometimes associated with FeLV. It occurs usually bilaterally and does not cause signs of illness until the kidneys are so extensively infiltrated that renal failure occurs. In these cases, kidneys are enlarged and usually irregular. Epidural lymphoma may cause sudden or gradual onset of posterior paralysis (Fig. 11-8).[297,413]

Leukemia may involve lymphoid cells (most common) but also all other hematopoietic cell lines. More than half of the cats with nonlymphoid leukemia have positive FeLV antigen test results. All hematopoietic cell lines are susceptible to transformation by FeLV, resulting in myeloproliferative disease or myelodysplastic syndrome (MDS; Figs. 11-9 and 11-10). Thus, lymphoid and myeloid (including granulocytic, erythroid, and megakaryocytic) types occur. The prognosis for cats with myeloproliferative diseases in general is poor. In acute

leukemia or MDS of any type, the bone marrow is filled with blast cells, and normal hematopoiesis is suppressed.[184] Clinical signs with acute leukemia are related to the loss of normal hematopoietic cells and include lethargy from anemia, signs of sepsis with neutropenia, and bleeding with thrombocytopenia. Hepatomegaly with icterus and splenomegaly are frequently present because of malignant infiltration or extramedullary hematopoiesis. Diagnosis of acute leukemia is made by CBC and bone marrow examination. Cytologic abnormalities of bone marrow include increased cellularity, megaloblastic maturation, increased myelofibrosis, and immature blast cells.[397] In cats with large numbers of circulating blast cells, the CBC may in itself be diagnostic. Although classifications have been proposed for the acute leukemias, the predominant cell type may be difficult to identify even with histochemical stains. Transformation, especially for the nonlymphoid leukemias, usually occurs at or very close to the stem-cell level, so more than one cell line may be affected. In some cats with acute leukemia, FeLV infection is found; a cat with a rare form of acute myelomonocytic leukemia and FeLV infection[320] and a cat with acute monoblastic leukemia and FeLV infection have been described.[353] A study focusing on acute myelocytic leukemia (AML) found that certain changes of the LTR of the FeLV in these cats may differ from the LTRs of other known FeLV strains in that it has three tandem direct 47-bp repeats in URE, and that FeLV variants that bear URE repeats in their LTR strongly associate with the induction of both MDS and AML in cats. The researchers injected cats with FeLV clone33 (originating from a cat with AML) and found that 41% of the cats developed MDS characterized by peripheral blood cytopenias and dysplastic changes in the bone marrow, and that some of the cats with MDS eventually developed AML. The bone marrow of the majority of cats with FeLV clone33-induced MDS produced fewer erythroid and myeloid colonies on being cultured with erythropoietin or granulocyte-macrophage colony-stimulating factor than bone marrow from normal control cats. Furthermore, the bone marrow of some of the cats expressed high levels of the apoptosis-related genes tumor necrosis factor (TNF)-α and survivin. Analysis of the proviral sequences obtained from 13 cats with naturally occurring MDS also found the characteristic URE repeats.[182] Chronic leukemias are rare in cats and rarely associated with FeLV. They include well differentiated chronic lymphocytic leukemia, chronic myelogenous leukemia, polycythemia vera, and thrombocythemia. In erythremic myelosis, proliferation of erythrocyte precursors is usually associated with FeLV-C, and most have positive test results for FeLV. Cats with this disorder have low hematocrit (HCT) levels (12% to 15%) with normal neutrophil counts and variable thrombocytopenia. The anemia is usually nonregenerative or poorly regenerative, and with time the HCT level does not increase. Despite the lack of regeneration, the mean corpuscular volume (MCV) and numbers of nucleated erythrocytes are usually high. Abnormal erythrocyte stages are found in bone marrow and often in peripheral blood. MDS may result as a clonal proliferation of hematopoietic cells that is a preleukemic state of acute myeloid leukemia.[183,400] Eosinophilic leukemia may be a subtype of chronic myelogenous leukemia and has been described in association with FeLV. A cat with chronic eosinophilic leukemia associated with FeLV infection has been published.[123] The differentiation between hypereosinophilic syndrome (severe reactive eosinophilia) and malignancy is difficult because both have been associated with large numbers of morphologically normal eosinophils in the marrow, peripheral blood, and other organs.[62,169]

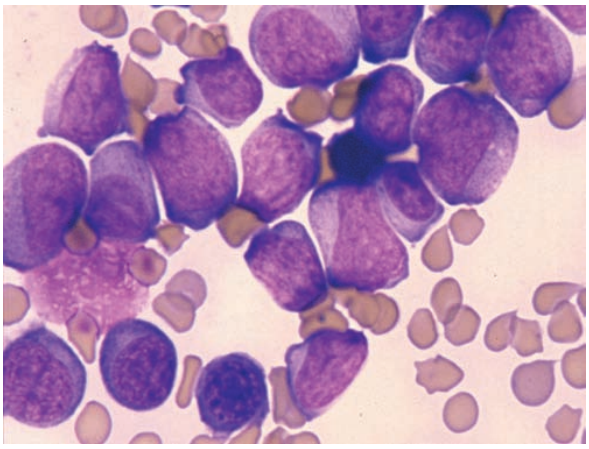

FIG. 11-9 Peripheral blood film of a cat with erythroleukemia. Cat had severe anemia without reticulocytosis. More than 95% of circulating nucleated cells were erythroid precursors of varying degrees of maturity. Severe granulocytopenia was noted (Wright stain, ×1000). (Photograph by Ken Latimer © 2004, University of Georgia Research Foundation Inc.)

Fibrosarcoma

Fibrosarcomas that are associated with FeLV are caused by FeSV, a recombinant virus that develops de novo in FeLV-A-infected cats by recombination of the FeLV-A genome with cellular oncogenes. Through a process of genetic recombination, FeSV acquires one of several oncogenes such as *fes, fms*, or *fgr.* As a result, FeSV is an acutely transforming (tumor-causing) virus, causing a polyclonal malignancy with multifocal tumors arising simultaneously after a short incubation period. With the decrease in FeLV prevalence, FeSV also has become less common. FeSV-induced fibrosarcomas are

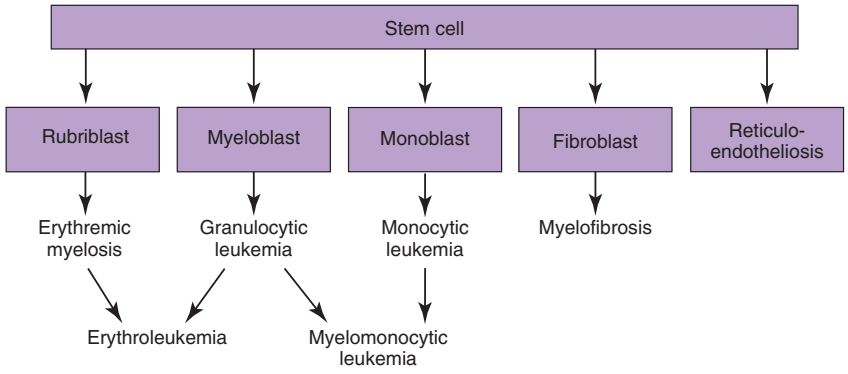

FIG. 11-10 Origin of cell lines in myeloproliferative disease. (Modified from Ref. 316; with permission.)

multicentric and usually occur in young cats. Several strains of FeSV that have been identified from naturally occurring tumors are defective. They are unable to replicate without the presence of FeLV-A as a helper virus that supplies proteins (such as those coded by the *env* gene) to FeSV. The host range for FeSV depends on the helper FeLV-A. By manipulation of the helper virus in the laboratory, FeSV can enter cells of species not naturally susceptible to infection. Experimental inoculation of FeSV has produced tumors in cats, rabbits, dogs, sheep, rats, and nonhuman primates.[431] Many of these tumors regress spontaneously, even after reaching a large size.[62] Fibrosarcoma cells express FOCMA just as lymphoma cells do. Experimental infection with FeSV causes tumors that progress in some cats and regress in others. Those in which the tumors regress have high FOCMA antibody titers.

Fibrosarcomas caused by various strains of FeSV tend to grow rapidly, often with multiple cutaneous or subcutaneous nodules that are locally invasive and metastasize to the lung and other sites. Solitary fibrosarcomas in old cats are not caused by FeSV. These tumors are slower growing, locally invasive, slower metastasizing, and occasionally curable by excision combined with radiation and/or gene therapy. These injection site–associated sarcomas are caused by the granulomatous inflammatory reaction at the injection site after inoculation of adjuvant-containing vaccines. It has been demonstrated that neither FeSV nor FeLV play any role in injection site–associated sarcomas.[94]

In addition to fibrosarcomas, FeSV has experimentally caused melanomas, showing that FeSV can transform cells of ectodermal and of mesodermal origin.[58] Intradermal or intraocular inoculation of FeSV into kittens produced melanomas in the skin or anterior chamber of the eye.[62] However, FeSV has not been associated with naturally occurring melanomas of cats.

Other Tumors

A number of other tumors have been found in FeLV-infected cats; some of them may have an association with FeLV, and some have been observed by chance simultaneously in an infected cat. Iris melanomas, for example, are not associated with FeLV infections as once was believed as a result of one study, in which 3 of 18 eyes had positive test results for FeLV-FeSV proviral DNA.[416] In a later study, however, immunohistochemical staining and PCR did not reveal FeLV or FeSV in the ocular tissues of any cats with this disorder.[67]

Multiple osteochondromas (cartilaginous exostoses on flat bones) have been described with increased prevalence in FeLV-infected cats. Although histologically benign, they may cause significant morbidity if they occur in an area such as a vertebra and put pressure on the spinal cord or nerve roots. The pathogenesis of these tumors is unknown.[276,350]

Spontaneous feline olfactory neuroblastomas are aggressive, histologically inhomogenous tumors of the tasting and smelling epithelium of nose and pharynx and have high metastasis rates. Budding FeLV particles were found in the tumors and lymph-node metastases, and FeLV DNA was found in tumor tissue.[389] Two of three cats described had positive FeLV antigen test results. The exact role of FeLV in the genesis of these tumors is uncertain.

Cutaneous horns are a benign hyperplasia of keratinocytes that have been described in FeLV-infected cats.[339] The exact role of FeLV in the pathogenesis is unclear.

Hematologic Disorders

Hematopoietic disorders, particularly cytopenias caused by bone marrow suppression, are a common finding in cats infected with FeLV (Table 11-4). Hematopoietic neoplasia ("myeloproliferative disorders"), including leukemia, may cause bone marrow suppression syndromes. In addition, a high percentage of infected cats develop nonneoplastic hematologic dysfunction. MDS, characterized by peripheral blood cytopenias and dysplastic changes in the bone marrow, is a pre-stage of AML. It was found that changes in the LTR of FeLV (presence of three tandem direct 47-bp repeats in the URE) are strongly associated with the induction of MDS.[182] Myelofibrosis, another cause of bone marrow suppression, is a condition characterized by abnormal proliferation of fibroblasts resulting from chronic stimulation of the bone marrow, such as chronic bone marrow activity from hyperplastic or neoplastic regeneration caused by FeLV. In severe cases, the entire endosteum within the medullary cavity can be obliterated. To diagnose this condition, a bone marrow core biopsy instead of needle aspiration is usually necessary.

Hematologic disorders described in association with FeLV include anemia (nonregenerative or regenerative); persistent, transient, or cyclic neutropenia; panleukopenia-like syndrome; platelet abnormalities (thrombocytopenia and platelet function abnormalities); and aplastic anemia (pancytopenia). For the majority of pathogenic mechanisms in which FeLV causes bone marrow suppression, active virus replication is required. However, it has been demonstrated that in some cats with negative antigen test results, regressive FeLV infection without viremia may be responsible for bone marrow suppression. In a study including 37 cats with myelosuppression that have positive FeLV antigen test results in peripheral blood, 2 cats (5%) were found regressively infected with FeLV by bone marrow PCR (both had nonregenerative anemia).[419] In these cats, FeLV provirus may interrupt or inactivate cellular genes in the infected cells, or regulatory features of viral DNA may alter expression of neighboring genes. Additionally, cell function of provirus-containing myelomonocytic progenitor and stromal fibroblasts that provide bone marrow microenvironment may be altered. Alternatively, FeLV provirus may cause bone marrow disorders by inducing the expression of antigens on the cell surface, resulting in an immune-mediated destruction of the cell.

Anemia

Anemia is a major nonneoplastic complication that occurs in a majority of symptomatic FeLV-infected cats.[126] In turn, it has been stated in the older literature that more than two thirds of all nonregenerative anemias in cats are the result of FeLV infection. As is the case with all FeLV-associated syndromes, this is clearly overestimated because of the decrease in overall FeLV prevalence. In a study investigating 79 anemic cats, FeLV was found in only 2 of 79 cats (both of them had hemolytic anemia).[243] Anemia in FeLV-infected cats may have various causes (see Table 11-4). Approximately 10% of FeLV-associated anemias are regenerative, indicated by a high reticulocyte count, high MCV, and presence of anisocytosis, nucleated erythrocytes, and polychromasia.[397] Regardless of the cause, regenerative FeLV-associated anemias usually have a favorable response to treatment. Most FeLV-associated anemias, however, are nonregenerative and caused by the bone marrow suppressive effect of the virus resulting from primary infection of hematopoietic stem cells and infection of stroma cells that constitute the supporting environment for hematopoietic cells. In vitro exposure of normal feline bone marrow to some strains of FeLV causes suppression of erythrogenesis.[62] In addition to the direct effect of the virus on erythropoiesis, other factors can cause nonregenerative anemia in FeLV-infected cats.

Hemolytic anemia caused by secondary infections (regenerative) may occur in FeLV-infected immunosuppressed cats. Clinical signs associated with hemolytic anemia are lethargy, anorexia, depression, pale mucous membranes, icterus, dehydration, and splenomegaly. The most common secondary infections responsible for hemolytic anemia in FeLV-infected cats are hemotropic *Mycoplasma* spp. infections (see Chapter 31). These organisms are not always found on

TABLE 11-4

Hematologic Disorders Related to Feline Leukemia Virus (FeLV) Infection

Causes	Mechanism	Hematologic Findings and Treatment
ANEMIA		
Hemolytic anemia caused by secondary infections (regenerative)	Virus-induced immunosuppression that allows hemotropic *Mycoplasma* species to replicate and cause disease	*Findings*: Regenerative anemia, variable icterus and hemoglobinemia, *Mycoplasma* spp. detected in blood smears or by or polymerase chain reaction (PCR) *Treatment*: Doxycycline
Immune-mediated hemolytic anemia (regenerative)	Virus-induced expression of foreign antigens on erythrocyte surface	*Findings*: Regenerative anemia (macrocytosis and reticulocytosis), variable icterus and hemoglobinemia, positive Coombs' test result *Treatment*: Immunosuppression (e.g., glucocorticoids)
Anemia of blood loss (regenerative)	Virus-induced suppression of platelet production by bone marrow or FeLV-associated platelet functional defects	*Findings*: Regenerative anemia, thrombocytopenia (<50,000 platelets/μL), low serum protein level *Treatment*: Blood transfusion and treatment of cause of thrombocytopenia
Pure red cell apalasia (PRCA) (nonregenerative)	Commonly FeLV-C infection; interaction of FeLV-C with cell surface receptors, blocking differentiation of erythroid progenitors between burst-forming units and colony-forming units by interfering with signal transduction pathways	*Findings*: Nonregenerative anemia with macrocytosis (high mean corpuscular volume [MCV]), other cell lines usually within reference ranges *Treatment*: Blood transfusion, may be responsive to immunosuppression
Anemia of chronic disease or anemia of chronic inflammation (nonregenerative)	Virus or secondary bacterial or neoplastic stimulation of inflammatory cytokines that sequester iron	*Findings*: Nonregenerative anemia *Treatment*: Removal or treatment of coexisting inflammatory disease or tumor; no response to erythropoietin
Anemia caused by crowding out (nonregenerative)	Lymphoma or leukemia as well as secondary infectious diseases, such as systemic mycosis or mycobacteriosis, leading to infiltration of the bone marrow and to "crowding out" bone marrow cells	*Findings*: Nonregenerative anemia *Treatment*: Removal or treatment of coexisting secondary infection or tumor
PURE PLATELET ABNORMALITIES		
Thrombocytopenia	Virus-induced immune-mediated thrombocytopenia or decreased platelet production from FeLV-induced bone marrow suppression or leukemic infiltration	*Findings*: Pure thrombocytopenia *Treatment*: In case of immune-mediated destruction immunosuppression (e.g., glucocorticoids) or treatment of the underlying disease (e.g., antitumor treatment)
Thrombocytopathy	FeLV replication in platelets leading to function deficits and shortened platelet life span, sometimes virus-induced neoplastic proliferation of megakaryocytes leading to thrombocytosis	*Findings*: Platelet function deficits (e.g., prolonged mucosal bleeding time), in case of neoplasia marked thrombocytosis (>600,000 platelets/μL) *Treatment*: In case of neoplasia, poor response to antitumor chemotherapy
PURE LEUKOCYTE ABNORMALITIES		
Lymphopenia	Destruction of lymphocytes through direct replication of the virus in lymphocytes	*Findings*: Pure lymphopenia *Treatment*: Antiviral chemotherapy
Neutropenia	Most likely virus-induced immune-mediated persistent or cyclic neutropenia, often after stressful episode	*Findings*: Pure neutropenia, may occur with or without a left shift; normal findings with all other cell lines *Treatment*: Immunosuppression (e.g., glucocorticoids)
Feline panleukopenia-like syndrome (FPLS), also called FeLV-associated enteritis (FAE), or myeloblastopenia	Likely caused by secondary feline panleukopenia virus (FPV) infection	*Findings*: Severe leukopenia (<3000 cells/μL) with enteritis and destruction of intestinal crypt epithelium with vomiting, diarrhea that mimics feline panleukopenia *Treatment*: Symptomatic treatment (see chapter 10) and treatment of overwhelming sepsis
PANCYTOPENIA		
Aplastic anemia or severe pancytopenia (nonregenerative)	Virus-induced (commonly FeLV-C) alteration of hematopoietic gene expression that affects early marrow precursor; affects multiple cell lines (near stem cell level)	*Findings*: Nonregenerative anemia, thrombocytopenia, leukopenia *Treatment*: Poor response to bone marrow stimulants or immunosuppressive therapy or bone marrow transplantation
Leukemia	Virus-induced neoplastic process involving leukocytes of myeloid or lymphoid cell lines	*Findings*: Nonregenerative anemia; commonly also neutropenia and thrombocytopenia, with large increase in lymphocytes or granulocyte precursors in peripheral blood *Treatment*: Poor response to antitumor chemotherapy

Continued

TABLE 11-4

Hematologic Disorders Related to Feline Leukemia Virus (FeLV) Infection—*Cont'd*

Causes	Mechanism	Hematologic Findings and Treatment
Myeloproliferative disease (erythroleukemia/ erythremic myelosis, reticuloendotheliosis, various granulocytic leukemias)	Virus-induced neoplastic transformation of erythrocyte precursors, granulocyte, or platelet precursors or stem cells, or all of these	*Findings*: Nonregenerative anemia, often with macrocytosis and variable numbers and types of nucleated erythrocytes, neoplastic cells in blood smears *Treatment*: Poor response to antitumor chemotherapy
Myelofibrosis	Abnormal proliferation of fibroblasts resulting from chronic stimulation of bone marrow, such as chronic bone marrow activity from hyperplastic or neoplastic regeneration caused by FeLV	*Findings*: Severe pancytopenia, entire endosteum within the medullary cavity obliterated, changes usually not diagnostic on bone marrow needle aspiration, core biopsy necessary *Treatment*: Poor prognosis, treatment of underlying condition

peripheral blood smears; however, diagnosis is possible with PCR techniques.[124,157]

FeLV-induced immune-mediated hemolytic anemia (IMHA; regenerative) also has been described. It is suspected that FeLV can induce an immune-mediated response leading to secondary IMHA with positive Coombs' test result, autoagglutination, and spherocytosis. IMHA occurs less frequently in cats than in dogs, but FeLV infection is a potential trigger. In a study on IMHA in cats, 2 of 19 cats had positive FeLV antigen test results.[237] However, in a more extensive study, Coombs' positive results in cats with anemia were not statistically associated with retrovirus or hemoplasma infection.[427a]

Anemia of blood loss (regenerative) may be present in a few cats with FeLV infection. It is usually seen in cats that have hemorrhage due to FeLV-associated thrombocytopenia or platelet functional defects.

Pure red cell aplasia (PRCA) (nonregenerative) is a severe isolated nonregenerative anemia (HCT below 15%) without regeneration. It can be caused by infection with FeLV-C through interactions of FeLV-C with cell surface receptors.[356,423] The cell surface receptor interactions block the differentiation of erythroid progenitors between burst-forming units and colony-forming units by interfering with signal transduction pathways essential for erythropoiesis.[355,397,463] Bone marrow examination shows an almost complete lack of erythroid precursors (at least late forms) with normal myeloid and megakaryocytic precursors and an increased myeloid-erythroid ratio.[62,257] These cats have typically have macrocytosis (rarely normocytosis) without evidence of reticulocyte response. Whenever macrocytic anemia (MCV greater than 60 fL) occurs in a cat in absence of reticulocytosis, FeLV infection should be suspected. Macrocytosis is caused through the FeLV-induced defect by skipped mitoses in cell division during erythropoiesis. These cats do not have folate or vitamin B_{12} deficiencies. Iron is present in macrophages but not erythrocyte precursors; however, iron kinetics are normal. Serum erythropoietin levels are markedly increased, indicating that anemia is not caused by an erythropoietin deficiency.[257] FeLV-associated PRCA is not a neoplastic or immune-mediated process because it is resistant to therapy. Treatment with immunosuppressive drugs (glucocorticoids and cyclophosphamide or ciclosporin) resulted in resolution of anemia within 3 to 5 weeks; however, relapse occurred when treatment was discontinued.[418]

Anemia of chronic disease or *anemia of chronic inflammation* (nonregenerative) is caused by excessive inflammatory cytokine production in FeLV-infected cats. It is characterized by a mild anemia (HCT of 20% to 30%). The HCT often increases spontaneously if the underlying problem is treated successfully, even if the cat continues to have positive test results for FeLV.

Anemia caused by crowding out (nonregenerative) is caused when infectious agents or neoplastic cells infiltrate the bone marrow and replace erythrocyte precursor cells. Lymphoma or leukemia as well as secondary infectious diseases, such as systemic mycosis or mycobacteriosis, may cause severe anemia by "crowding out" bone marrow cells.

Aplastic anemia or severe *pancytopenia* (nonregenerative) may be present in FeLV-infected cats and involves all cell lines. Bone marrow cytology is usually hypocellular or may show necrosis.[401] Cats with pancytopenia often had positive test results for FeLV antigen in earlier times, but in a study of 13 cats with aplastic anemia from 1996 to 2004, only 2 of 13 were found to be have positive FeLV antigen test results.[456] In this condition, the virus probably affects precursors near the stem cell level. In some cats, cyclic hematopoiesis with periodic fluctuation in reticulocytes, granulocytes, and platelets may be noted. Alteration of accessory cells within the bone marrow microenvironment providing the structural framework, cytoadhesive molecules, and growth-regulatory cytokines necessary for normal hematopoiesis may be the cause. FeLV can affect bone marrow accessory cell viability, growth, production, or all of these of hematopoietic progenitor growth-regulating substances by altering cytokine mRNA levels in general and strain-specific patterns.[266–268] In bone marrow cytology, few if any precursors may be found, and core biopsy specimens may be needed. The aplastic marrow may represent a more advanced stage of myelosuppression than PRCA. Bone marrow transplantation or immunosuppression has not been successful in these cats.

Platelet Abnormalities

FeLV infection can cause decreased platelet counts. It also may be responsible for platelet function deficits.

Thrombocytopenia may occur secondary to decreased platelet production from FeLV-induced bone marrow suppression or leukemic infiltration. The life span of platelets is shortened in some FeLV-infected cats. Platelets harbor FeLV proteins as a result of infection. In addition, megakaryocytes, the marrow precursors of blood platelets, are frequent targets of progressive FeLV infection. Immune-mediated thrombocytopenia, which rarely occurs as a single disease entity in cats, often accompanies IMHA in cats with underlying FeLV infection. Thrombocytopenia may result in bleeding tendencies.

Thrombocytopathy in FeLV-infected cats involves platelet changes not only in quantity, but also in size, shape, and function. FeLV replicates in platelets and may alter platelet function. The life span of

platelets is shortened in some FeLV-infected cats. Giant platelets and thrombocytosis have been observed in some progressively infected cats.[397]

Leukocyte Abnormalities

FeLV-infected cats may have decreased neutrophil or lymphocyte counts or impaired function. In addition, a so-called feline panleukopenia-like syndrome (FPLS) has been described in FeLV-infected cats.

Lymphopenia is primarily a result of direct replication of the virus in lymphocytes. Affected cats may develop thymic atrophy and depletion of lymph node paracortical zones after infection. In some cats, lymphopenia may be characterized by preferential loss of $CD4^+$ helper T cells, resulting in an inverted CD4/CD8 ratio.[355] More commonly, substantial losses of helper cells and cytotoxic suppressor cells (CD8+ cells) occur.[185]

Neutropenia is common in FeLV-infected cats[38] and generally occurs alone or in conjunction with other cytopenias. In some cases, myeloid hypoplasia of all granulocytic stages is observed, suggesting direct cytopathic infection on neutrophil precursors by FeLV. In some neutropenic FeLV-infected cats, an arrest in bone marrow maturation may occur at the myelocyte and metamyelocyte stages. It has been hypothesized that an immune-mediated mechanism is responsible in cases in which neutrophil counts recover with glucocorticoid treatment ("glucocorticoid-responsive neutropenia"). Cyclic neutropenia also has been reported in cats with FeLV infection and usually is effectively treated with glucocorticoids, suggesting that immune-mediated mechanisms are also likely in this syndrome. The cycles are usually regular, ranging from 8 to 14 days. Bone marrow cytology during the neutropenic phase may indicate either granulocytic hyperplasia or hypoplasia, with a disproportionate number of cells in the promyelocytic stage. Similar bone marrow findings could result from inflammatory or immune-mediated diseases, myelodysplasia, or granulocytic leukemia. Cats with neutropenia usually have recurrent fever or persistent bacterial infections. Some cats show persistent gingivitis, occasionally without the usual signs of inflammation such as hyperemia and purulent exudate because granulocytes are necessary for the inflammatory response.[62] In addition to problems associated with low neutrophil counts, neutrophils of progressively infected cats may have decreased chemotactic and phagocytic function.

FPLS, also known as *FeLV-associated enteritis (FAE)* or *myeloblastopenia*, consists of severe leukopenia (fewer than 3000 cells/μL) with enteritis and destruction of intestinal crypt epithelium that mimics feline panleukopenia caused by feline panleukopenia virus (FPV) infection (see Chapter 9). FPV antigen has been demonstrated by immunofluorescence in intestinal sections of cats that died from this syndrome after being experimentally infected with FeLV.[280] FPV was also demonstrated by electron microscopy despite negative FPV antigen tests. It appears that this syndrome may actually not be caused by FeLV itself, as previously thought, but by co-infection with FPV. The syndrome also has been referred to as FAE in cats with progressive FeLV infection because the clinical signs observed are usually GI, including hemorrhagic diarrhea, vomiting, oral ulceration or gingivitis, anorexia, and weight loss.[230,231] It is still unclear whether all theses syndromes are simply caused by co-infection with FPV (and even modified live FPV vaccines have been discussed) or if they are caused by FeLV itself.[280] In experimental studies, a similar syndrome could be induced, leading to enteritis with proliferation of FeLV antigen within the enterocytes, when cats had been experimentally infected with FeLV-FAIDS variants of FeLV. FeLV FAIDS infection begins with a prodromal period of lymphoid hyperplasia associated with viral replication in lymphoid follicles, followed by lymphoid depletion associated with extinction of viral replication. Cats develop enterocolitis with crypt necrosis and villous atrophy.[193] Intractable diarrhea and weight loss are associated with immunodeficiency characterized by lymphopenia, suppressed lymphocyte stimulation, impaired cutaneous allograft rejection, hypogammaglobulinemia, and opportunistic infections such as respiratory disease or stomatitis. These observations suggest that the development of FPLS and/or FAE may be FeLV strain-dependent.

Immunosuppression

Diseases secondary to immunosuppression account for a large portion of the morbidity and mortality of FeLV-infected cats.[84,330,337] Progressively FeLV-infected cats are predisposed to secondary infections primarily because of immunosuppression similar to that in human patients infected with human immunodeficiency virus (HIV), but immunosuppression is more severe than the one caused by FIV infection. Evaluation of the true immune status of FeLV-infected cats is hampered by the lack of well-characterized tests. Thus, clinicians primarily depend on CBC and clinical presentation for diagnosing immune dysfunction. Some commercial laboratories offer selective counts of CD4+ and CD8+ cells, but the value of these parameters rarely has been evaluated in naturally infected cats.[185]

The exact mechanisms by which the virus damages the immune system are poorly understood, as is why different animals have such varying degrees of immunosuppression in response to the same virus. Immunosuppression is occasionally associated with unintegrated viral DNA from replication-defective viral variants.[333] These pathogenic immunosuppressive variants, such as FeLV-T, require a membrane-spanning receptor molecule (Pit1) and a second co-receptor protein (FeLIX) to infect T lymphocytes.[251] The latter protein is an endogenously expressed protein encoded by an endogenous provirus arising from FeLV-A, which is similar to the FeLV receptor-binding protein of FeLV-B.[24] Affected cats may develop thymic atrophy and depletion of lymph node paracortical zones after infection. Lymphopenia and neutropenia are common. In addition, neutrophils of viremic cats have decreased chemotactic and phagocytic function compared with those of normal cats. This abnormality persists for an unknown period, even if viremia is transient. In some cats, lymphopenia may be characterized by preferential loss of CD4+ helper T cells, resulting in an inverted CD4/CD8 ratio (which is more typical of FIV infection).[185,355] More commonly, substantial losses of helper cells and cytotoxic suppressor cells (CD8+ cells) occur.[185] Many immune function tests of naturally FeLV-infected cats have been reported to be abnormal, including poor response to T-cell mitogens, prolonged allograft reaction, reduced immunoglobulin production, depressed neutrophil function, and complement depletion. Interleukin (IL)-2 and IL-4 are decreased in some cats.[257,267] However, studies disagree on whether interferon (IFN)-γ is deficient or increased. FeLV does not appear to suppress (IL-1 production from infected macrophages. Increased TNF-α has been observed in serum of infected cats and infected cells in culture. Although each cytokine plays a vital role in the generation of a healthy immune response, the excess production of certain cytokines such as TNF-α can also cause illness.

Primary and secondary humoral antibody responses to specific antigens are delayed and decreased in FeLV-infected cats. In vaccination studies, FeLV-infected cats have not been consistently able to mount an adequate immune response to vaccines such as rabies. Therefore, protection in a FeLV-infected cat after vaccination is not comparable to that in a healthy cat, and more frequent vaccinations (e.g., every 6 months) have to be considered. T cells of FeLV-infected cats produce significantly lower levels of B-cell stimulatory factors than do those of normal cats.[84] This defect becomes progressively more severe over time. However, when B cells of FeLV-infected cats

are stimulated in vitro by uninfected T cells, their function remains normal. Although humoral immunity to specific stimulation decreases, nonspecific increases of IgG and IgM have been noted.

Immune-Mediated Diseases

In addition to immunosuppression, FeLV-infected cats are subject to various immune-mediated diseases caused by an overactive or dysregulated immune response to the virus. FeLV-associated immune-mediated diseases including autoimmune hemolytic anemia,[237] glomerulonephritis,[9] uveitis with immune complex deposition in iris and ciliary body,[35] and polyarthritis.[339] Chronic progressive polyarthritis can be triggered by FeLV; in about 20% of cats with polyarthritis, FeLV seems to be an associated agent.[339] A similar syndrome can also be caused by feline foamy virus and concurrent FIV infection may occur in either instance (see Chapter 15).[326a]

The loss of T-suppressor cell activity and the formation of antigen-antibody complexes contribute to these immune-mediated diseases.[338] Measurement of FeLV antigens has shown that cats with glomerulonephritis have more circulating viral proteins that do other FeLV-infected cats, although in a study, FeLV-infected cats in general did not show significantly more commonly hypergammaglobulinemia in plasma electrophoretogram in contrast to FIV-infected cats,[309] and hyperproteinemia is not a common problem in FeLV-infected cats (different from FIV infection).[127] Antigens that can lead to antigen-antibody complex formation include not only whole virus particles but also free gp70, p27, or p15E proteins.[76,440] Circulating immune complexes (CICs) have also been observed after experimental treatment of persistent viremia with MABs to gp70 and in studies of inoculation of complement-depleting factors.

Other Syndromes

Other syndromes directly caused by FeLV infection include reproductive disorders, fading kitten syndrome, and FeLV-associated neuropathy. Beside those syndromes, other clinical signs observed are likely the result of secondary infections, and from a clinical standpoint, it is important to realize that many of these secondary diseases are treatable. Many reports have been made of FeLV-infected cats having concurrent bacterial, viral, protozoal, and fungal infections, but few studies exist proving that these cats have a higher rate of infection than do FeLV-negative cats or that they have a less favorable response to therapy. Thus, although FeLV is well known to suppress immune function, it should not be assumed that all concurrent infections are a result of FeLV infection. Secondary infections that may be associated with FeLV include FIP, coccidiosis, and upper respiratory infections.[257,361,362] Studies have focused on the role and the influence of FeLV infection on hemotropic *Mycoplasma* spp. infection with controversial results, because in some studies, *Mycoplasma* infection was associated with FeLV infection,[26,422] whereas in others it was not.[244,284,459] No association was found with leishmaniasis in cats.[295] Chronic ulcerative stomatitis also was not associated with FeLV infection in two studies.[28,357] Cats that were naturally infected with FeLV were more likely to develop and not eliminate *Bartonella henselae* infection; however, the course and clinical outcome of bartonellosis was not different in cats that were not co-infected with FeLV.[38a]

Other diseases also can be indirectly influenced by FeLV, such as a hepatopathy described in FeLV-infected cats with icterus and various inflammatory and degenerative liver diseases.[361,362] Hepatic lipidosis is a major complicating factor that can explain some of these cases; however, unexplained focal liver necrosis was also observed. Skin disease has been described in some FeLV-infected cats. FeLV-infected cats have a greater diversity of cutaneous and mucosal microflora compared with uninfected cats,[403] and infections associated with dermatologic conditions are usually caused by the immunosuppression.[347] Traumatic injuries are complicated by secondary bacterial infections or abscesses. Otitis externa and miliary dermatitis may develop from ectoparasites or allergies but persist because of secondary bacterial infections.

Reproductive Disorders

FeLV-infected queens can transmit the virus transplacentally. Reproductive failure in the form of fetal resorption, abortion, and neonatal death is common if in utero FeLV infection occurs. The apparent infertility might actually be early resorption of fetuses. Abortions usually occur late in gestation, with expulsion of normal-appearing fetuses. Bacterial endometritis may accompany these abortions, particularly in cats with neutropenia.[62]

Fading Kitten Syndrome

Kittens born to infected queens may become exposed to FeLV transplacentally, but heavy exposure also occurs at birth and throughout the nursing period. Some kittens become immune, but most become progressively infected and die at an early age of the so-called fading kitten syndrome, characterized by failure to nurse, dehydration, hypothermia, thymic atrophy, and death within the first 2 weeks of life.[257]

Neuropathy

Neurologic dysfunction has been described in FeLV-infected cats. Although most neurologic signs seen in FeLV-infected cats are caused by lymphoma and lymphocytic infiltrations in brain or spinal cord leading to compression, in some cases no tumor is detectable with diagnostic imaging methods or at necropsy. Anisocoria, mydriasis, central blindness, or Horner's syndrome have been described in FeLV-infected cats without morphologic changes. In some regions (such as the southeastern United States), urinary incontinence caused by neuropathies in FeLV-infected cats has been described.[44] Direct neurotoxic effects of FeLV have been discussed as pathogenetic mechanisms. Envelope glycoproteins of retroviruses may be able to produce increased intracellular free calcium leading to neuronal death, as observed in HIV-infected humans. A polypeptide of the FeLV envelope was found to cause dose-dependent neurotoxicity associated with alterations in intracellular calcium ion concentration, neuronal survival, and neurite outgrowth. The polypeptide from an FeLV-C strain was significantly more neurotoxic than the same peptide derived from an FeLV-A strain.[104,310]

Clinical signs in 16 cats with progressive FeLV infection and neurologic signs consisted of abnormal vocalization, hyperesthesia, and paresis progressing to paralysis. Some cats developed anisocoria or urinary incontinence during the course of their illness. Others had concurrent FeLV-related problems such as myelodysplastic disease. The clinical course of affected cats involved gradually progressive neurologic dysfunction. Microscopically, white-matter degeneration with dilation of myelin sheaths and swollen axons was identified in the spinal cords and brainstems of affected animals.[44] Immunohistochemical staining of affected tissues revealed consistent expression of FeLV p27 antigens in neurons, endothelial cells, and glial cells, and proviral DNA was amplified from multiple sections of spinal cord.[44] These findings suggest that in some FeLV-infected cats, the virus may directly affect CNS cells cytopathically.

DIAGNOSIS

Testing for FeLV and consequently preventing exposure of healthy cats to FeLV-infected cats is the most effective way to prevent the spread of infection. Testing to identify infected cats is the mainstay of preventing transmission, and FeLV vaccination should not be

considered a substitute for testing. The American Association of Feline Practitioners (AAFP) has established guidelines for testing cats for FeLV.[260] According to these guidelines, the FeLV status of all cats should be known, because infection has serious health consequences that influence patient management, both in illness and for wellness care. Accurate diagnosis of infection is important for both uninfected and infected cats. Identification and segregation of infected cats is considered to be the most effective method for preventing new infections in other cats. Failure to identify infected cats may lead to inadvertent exposure and transmission to uninfected cats. Misdiagnosis of infection in uninfected cats may lead to inappropriate changes in lifestyle or even euthanasia.[260] To completely eliminate any risk to an established household when bringing in a new cat, a follow-up test should be performed at least 90 days after the initial test or after a possible exposure to FeLV because cats may be in the early stage of infection at the time of the first test; the test should be performed before bringing the cat into the home.[255]

Cats can be tested at any age. Because the screening tests detect antigen and not antibodies, neither maternal antibodies nor antibodies from vaccination or previous viral exposure interfere with testing. It has to be realized, however, that kittens infected by some form of maternal transmission may not test positive for weeks to months after birth.[259] Vaccination against FeLV does not generally compromise testing, because FeLV tests detect antigen and not antibodies. However, blood collected immediately after vaccination may contain detectable FeLV antigens from the vaccine itself, so diagnostic samples should be collected before FeLV vaccine administration.[260] It is not known how long this test interference persists. Cats may require retrovirus testing at different times in their lives; for example, cats that meet certain criteria (Box 11-1) should be tested for FeLV infection.[260]

BOX 11-1

Criteria for Testing Cats for FeLV Infection[a]

- Sick cats (even if they have tested negative in the past).
- Newly acquired cats and kittens.
- Even cats that do not live with other cats should be tested for several reasons. Their FeLV status may influence their health, other cats may join the household in the future, or cats confined indoors may escape and expose other cats.
- Tests should be performed at adoption, and cats with negative results should be retested in a minimum of 28 days.
- Cats with known recent exposure to a FeLV-infected cat or to a cat with unknown status, such as via a bite wound.
- Testing should be carried out immediately, and if negative should be repeated in a minimum of 28 days.
- Cats living in households with other cats infected with FeLV should be tested on an annual basis unless they are isolated.
- Cats with high-risk lifestyles should be tested on a regular basis (e.g., cats that have access to outdoors in cat-dense neighborhoods and cats with evidence of fighting such as bite wounds and abscesses).
- Cats should be tested before initial vaccination against FeLV.
- Cats used for blood or tissue donation should have negative screening test results for FeLV in addition to negative real-time PCR test results.
- Intermittent retesting is not necessary for cats with confirmed negative infection status unless they have an opportunity for exposure to infected cats or if they become ill.

[a]From Ref. 260, with permission.

Direct Detection of the Virus

For the diagnosis of FeLV infection, usually direct methods of virus detection are preferred because routine tests are available that detect free FeLV p27 antigen (produced abundantly by virus infected cells) in blood. Direct FeLV detection methods include detection of free (by ELISA or other immunochromatographic methods) or cell-bound (by direct FA) FeLV antigen, detection of viral nucleic acid by PCR including detection of provirus (DNA) or virus (RNA), and virus isolation.

Detection of FeLV Antigens

Routine screening for FeLV became available with the development of immunofluorescence assays testing for virus in 1973.[150] In 1979, the first commercial ELISA was licensed. It was very sensitive in detecting low concentrations of antigen in serum of infected cats,[274] but it was not very specific. Lutz and others[282] developed an ELISA containing MABs against three different epitopes of p27 antigen that did not cross-react with proteins of other retroviruses; thus, the resulting test was more specific. Several ELISAs and other immunochromatographic assays (ICGAs) or rapid immunomigration assays are used. The membrane-fixed ICGAs and rapid immunomigration assays are based on a principle similar to ELISA in which color is generated as a result of an immunologic reaction, but the assays have a slightly different design than ELISA. All ELISA-based methods are available for use as rapid point-of-care tests.[371]

The colorimetric point-of-care ELISA-based assays are the mainstay of clinical testing today, but direct FA testing for viral antigen is still in use.[153] Direct FA and ELISA-based methods both detect FeLV core protein p27, which is produced abundantly in most infected cats; however, ELISA-based methods are more sensitive and detect lower levels of free soluble FeLV p27 in plasma or serum, whereas direct FA only detects larger quantities of p27 antigen within the cytoplasm of infected blood cells. Both ELISA-based methods and direct FA are useful clinically. Cats that *only* have positive ELISA-based test results are more likely to later regress to negative results than are cats with positive results on *both* ELISA-based and direct FA tests. To distinguish between regressive and progressive infection, cats should be retested with ELISA-based methods 6 weeks after the first positive test result. If a cat still has a positive result, it should be retested after another 10 weeks. If at this time the cat still has a positive result, it is most likely progressively infected and will have positive results for the rest of its life. Another method without the retesting delay is to immediately test a cat with a positive ELISA-based method results with direct FA. If the direct FA result is positive, the likelihood of a transient viremia (regressive infection) is small. Only 3% to 9% of cats with positive direct FA results have a transient viremia.[146,153,212,216,282] A small number of cats with discordant test results that develop persistently positive results with ELISA-based methods, and negative results with direct FA methods, may have focal or atypical infections that are kept localized by their immune systems.[216] A negative ELISA-based method result but positive direct FA method result is always a false result, either a false-negative ELISA result (which is very unlikely), or more likely, a false-positive direct FA result.

ELISA-based methods detect free soluble FeLV p27 and are the recommended screening tests for FeLV infection. A positive whole-blood, serum, or plasma ELISA-based method means that the cat is viremic. These tests become positive early, in the first phase of viremia within the first weeks after infection, before the bone marrow is affected. Thus, positive results may be reflective of transient viremia (in regressively infected cats) or persistent viremia (in progressively infected cats).[25] In experimental settings, most cats have positive results within 28 days after exposure.[212] Even the improved

ELISA-based methods can have false-positive results for numerous reasons. Although they can be performed on serum, plasma, or whole blood, in some studies, higher rates of false-positive results were recorded when whole-blood samples were used, particularly when the samples were hemolyzed. Thus, standard ELISA methods should *only* be performed with plasma or serum. However, the ICGA-based tests contain a filtering membrane, so whole blood and serum and plasma do not produce different results.[170] False-positive results were also a problem in some test systems that used murine-derived reagents in cats that had naturally occurring anti-mouse antibodies,[274] which are present in about 1% to 2% of all cats. Improved tests have solved that problem by including additional control steps. Technical and user errors contribute to false-positive results as well.[153,285] These errors are most likely to occur during the washing steps of kits using microwell or plate formats. Membrane-based tests eliminate separate washing steps and include positive and negative controls for each test sample. Comparative studies have been performed on many ELISA-based tests since they began to be marketed, especially in Europe.[168,170,345,384] In the majority of these studies, sensitivities and specificities were comparable. Positive predictive values of most tests were about 80%,[168,170] whereas negative predictive values were close to 100%.[136,170] The reliability of a test (its predictive values) depends on the rate of infection within a cat population. False-positive results are more important today because the decreasing prevalence of FeLV is leading to lower positive predictive values of the available tests. Thus, because FeLV is present in most cats with thymic lymphoma, a positive test result is likely to be accurate in this situation, whereas in a lower-risk population, such as a closed cattery known to be free of FeLV, a positive test should be viewed with more suspicion, and confirmatory tests should be performed.[257] Therefore, negative test results are highly reliable because of the low FeLV prevalence in most populations, but positive results have to be interpreted carefully, and confirmatory tests have to be considered after a positive result. If confirmatory tests (e.g., virus isolation, PCR) are not available or are too expensive to perform, at the very least a second ELISA-based test should be performed to rule out a false-positive result. If the second test is positive, this significantly increases the predictive value.[170] Retesting should be performed immediately and has nothing to do with the different stages of viremia; it is only used to compensate for the weaknesses of the test systems. Some ELISAs have been developed for tear and saliva samples in place of blood.[171] In general, these tests are not as accurate as blood testing because antigen shedding is intermittent and the tests are subject to more technical errors[18,171]; they are not recommended because the consequences for false-negative and false-positive results can be disastrous for individual cats or multiple-cat populations.[29,171,172,281]

Direct FA testing on smears from blood or bone marrow detects cell-associated p27 antigen within infected blood cells, primarily in neutrophils and platelets. The earliest the test becomes positive after infection of the bone marrow is after at least 3 weeks of viremia (secondary viremia). Positive test results are likely to reflect persistent viremia (progressive infection)[147,149,257]; therefore, direct FA testing is not recommended as a screening test because cats that are in the first weeks of viremia, but already infectious to others, are not detected. Direct FA testing can be used for prognostic reasons or to confirm positive and suspicious results. Direct FA methods require special processing and fluorescent microscopy and must be performed by a qualified reference laboratory. Refer to Web Appendix 5 for a list of qualified labs performing these tests. Generally, two or more quality blood smears should be air dried and mailed, unfixed, to the laboratory. As antigen is present at highest concentrations in neutrophils and platelets, false-negative results may occur when these two cell lines are deficient. False-positive results occur when smears are too thick, background fluorescence is high, or the test is prepared and interpreted by inexperienced personnel. Using anticoagulated blood rather than fresh blood for making smears can also cause errors.[209,454] Variations in quality control among facilities have been reported, and careful attention should be paid to the selection of the reference laboratory.[257]

Nucleic Acid Detection

PCR differs from direct FA and ELISA-based methods in that it does not detect viral antigen (protein) but viral nucleic acid sequences (viral RNA or proviral [cell-associated] DNA). It can be performed on blood, bone marrow, and tissues. PCR is a very sensitive method because the process involves amplification of FeLV gene sequences to enhance detection. PCR testing is offered by a number of commercial laboratories (see Web Appendix 5). When performed under optimal conditions, PCR can be the most sensitive test methodology for FeLV diagnosis and can help resolve cases with discordant antigen test results. However, PCR must be performed by well-equipped and well-trained laboratories because minor alterations in sample handling can destroy the delicate nucleic acid material or introduce minute amounts of cross-contamination, leading to either false-negative or false-positive results, respectively. Technical errors can reduce sensitivity and specificity of PCR results significantly. There are no comparative studies of the diagnostic accuracy of various commercial laboratories offering FeLV PCR. In addition, PCR is highly strain specific. As a retrovirus, FeLV mutates naturally, and minor strain variations may prevent binding of the primers, a step necessary to amplify the viral genome. Cats infected with mutated FeLV may have negative test reactions with a specific PCR. Thus, a negative result does not necessarily mean that a cat is uninfected. PCR is most accurate if it reveals a positive result and if it is performed by a reputable laboratory so that contamination can be excluded. With this in mind, PCR has greatly enhanced the possibilities of detecting FeLV infection in blood, cultures, solid tissue, and fixed specimens.

The main indication for PCR is the suspicion of a regressive (latent) infection in cats with lymphomas, bone marrow-suppressive syndromes, or chronically inflamed oral gingival lesions.[174,204,419,442] In regressive infection, no or minimal replication virus is present; thus, tests such as ELISA/ICGA that detect viral antigen are negative. In addition, real-time PCR is used to quantify provirus and virus loads.[14,48,49,346,437] Using quantitative (real-time) PCR, it has been shown that viral loads in experimentally infected cats with negative ELISA test results (i.e., that were regressively infected) that mount an effective immune response were much lower (300-fold less) than viral loads in cats with positive antigen test results (i.e., that were progressively infected).[189] If quantitative PCR is used to investigate proviral and viral RNA loads in leukocyte subsets, it also might allow differentiation of regressive and progressive FeLV infection.[47] Furthermore, studies using real-time PCR found that 5% to 10% of cats with negative antigen tests were positive for FeLV provirus by PCR.[131,189] Although the clinical significance of antigen-negative, provirus-positive status is still unknown, it appears that most of these cats remain aviremic, do not shed virus, and are unlikely to ever develop FeLV-associated diseases. PCR of bone marrow samples in cats with myelosuppression[419] and of tumor tissue samples from cats with lymphoma have demonstrated regressive FeLV infection in FeLV antigen-negative cats.[167,204,206] Rates of detection are greater in bone marrow than in blood from regressively infected cats that have negative antigen test results.[419] Thus, ideally samples should be taken from bone marrow, lymph node aspirates, or neoplasms rather than blood.

It is also possible to detect virus shedding in saliva using sensitive PCR methods. A study of the shedding pattern of FeLV RNA in saliva

found that active shedding was a consistent feature in progressively infected cats, whereas regressively infected cats with a low proviral load did not shed viral nucleic acid in saliva. FeLV RNA and DNA were stable for more than 64 days in saliva samples stored at room temperature, and in naturally infected cats, a high sensitivity and specificity of tests on saliva was found when compared to tests for antigen in blood.[131,132] The authors suggested that detection of salivary viral RNA by PCR could become a reliable noninvasive tool for the diagnosis of FeLV infection. In another study,[97] field cats were identified that had positive FeLV antigen test results in blood but negative DNA and RNA PCR test results using saliva. These results suggest that some PCR methods on saliva may not be sufficiently sensitive to replace blood testing, at least in the near future.

Virus Isolation

Virus isolation was originally developed to identify FeLV-infected cats.[82,213] It is not practicable for routine diagnosis because it is difficult and time-consuming to perform and requires special facilities. It may still be used for the confirmation of positive test results and suspicious samples.

Antibody Detection Methods

Detection of antibodies is not useful to diagnose FeLV infection, because many cats immune to FeLV have antibodies, whereas progressively infected cats have no detectable antibodies. Antibodies and immunity will follow vaccination or regressive or abortive infection. Some of these immune cats indeed will have FeLV infection (e.g., regressive infection), but others will not (e.g., vaccinated cats), and antibody testing systems do not distinguish between antibodies caused by vaccination and by natural infection. Moreover, many cats may be vaccinated and regressively infected simultaneously, because a study has shown that vaccination does not prevent infection.[187] Experimentally it has been shown that FeLV low-dose exposure can result in antibody production during an abortive infection, with cats having anti-FeLV antibodies, but no FeLV antigen or nucleic acid detectable.[292]

On the other hand, antibody testing may predict immunity against FeLV infection, and thus, cats with antibodies against FeLV are unlikely to benefit from FeLV vaccination. The connection between presence of antibodies and immunity, however, is not absolute, because many vaccinated cats will not develop antibodies,[97] and there will be cats that are protected against FeLV despite the presence of detectable antibodies.[248,412] Antibody testing may help to reveal the FeLV status of a population. In a study assessing the status of FeLV infection in the cat population of southern Germany, many cats were found to have FeLV antibodies despite having negative antigen and PCR test results.[97]

THERAPY

Despite the fact that persistent FeLV viremia is associated with a decreased life expectancy, many owners elect to provide treatment for the myriad clinical syndromes that accompany infection. Some older studies suggested that FeLV-infected cats live only a maximum of 3 years after diagnosis, but these studies involved group-housed cats in multiple-cat, FeLV-endemic environments. With proper care, FeLV-infected cats may live much longer than 3 years and, in fact, may die at an older age from causes completely unrelated to their retroviral infection.[160] Thus, decisions about treatment or euthanasia should never be based solely on the presence of FeLV infection. It is important to realize that FeLV-infected cats are subject to the same diseases that befall uninfected cats, and the mere presence of an FeLV-related disease may or may not be caused by FeLV.[162,260]

Management of Feline Leukemia Virus-Infected Cats

Special management has to be considered when owning a FeLV-infected cats. These management protocols must include the housemates of the FeLV-infected cat.

Feline Leukemia Virus–Infected Households

In a household with an FeLV-infected cat, all cats should be tested so that their status is known. If one or more cats with negative FeLV antigen test results are identified in a household with FeLV-infected cats, the owners must be informed of the potential danger to the uninfected cats in the house. They should be told that the best method of preventing the spread of infection is to isolate the infected individuals in other rooms to keep the infected cats from interacting with uninfected housemates. Shedding of virus generally occurs through salivary glands, and cat-to-cat transmission can occur by allogrooming, sharing of food and water bowls and litter boxes, and fighting and biting behavior. The risk of transmission is not very high because the cats that have lived together with FeLV-shedding cats have already been exposed or infected and are more likely to be immune to new infection. However, studies in cluster households have shown that virus neutralization is not lifelong; therefore, a previously immune cat can become viremic, which may reflect reactivation of a regressive infection. However, truly new infections (although unlikely) cannot totally be ruled out.[162] The risk that an adult cat previously having a negative FeLV antigen test result will develop a positive test result is approximately 10% to 15% if the cat has lived with a shedding cat for more than several months.[59] If owners refuse to separate housemates, the uninfected cats should receive FeLV vaccination in an attempt to enhance their natural level of immunity in this environment of high viral exposure. However, vaccination does not provide good protection under these circumstances. If the household is closed to new cats, the cats with negative FeLV antigen test results will tend to outlive the progressively infected cats; thus, after months or years all remaining cats will be immune.

Individual Feline Leukemia Virus-Infected Cats

FeLV-infected cats should be confined indoors, not only to prevent spread to other cats in the neighborhood but also to protect the vulnerable immunosuppressed cats from other infectious agents carried by other animals. Good nutrition and husbandry are essential to maintain good health. FeLV-infected cats should be fed a high-quality commercial feline diet. Raw meat, eggs, and unpasteurized milk should be avoided because of the risk of acquiring foodborne bacterial or parasitic infections.[255]

Wellness visits to the veterinarian should occur at least semiannually to promptly detect changes in health status. Visits should include a detailed history, a thorough physical examination with special attention to palpation of lymph nodes, examinations of the oral cavity to detect dental and gum diseases and the skin to detect external parasite infestation or fungal disease, an ophthalmic examination to investigate the anterior and posterior segments of both eyes, and an accurate measurement and recording of the body weight as marker for the cat's general condition. In addition, a CBC should be performed at each visit, and a biochemistry profile, urinalysis (including a bacterial culture), and fecal examinations (in cats with possible exposure or a history of GI problems) at least yearly. Intact males and females should be neutered to reduce stress associated with estrus and mating behavior and to decrease the desire to roam outside. Surgery is generally well tolerated by asymptomatic FeLV-infected cats. Perioperative antibiotic administration should be used during surgeries and dental procedures.[255,260]

Vaccination with core vaccines (against FPV, feline herpesvirus, and feline calicivirus) should be performed regularly, even if the cat is kept strictly indoors. If an owner cannot be convinced to keep a FeLV-infected cat inside, a rabies vaccination should be given (in accordance with state and local regulations). FeLV-infected cats may not be able to mount an adequate immune response to administered vaccines, which has been observed for rabies vaccines but is likely for other vaccines as well. Therefore, protection in a FeLV-infected cat after vaccination is not as complete and long-lasting as in a noninfected cat, and more frequent vaccinations (e.g., every 6 months) have to be considered in FeLV-infected cats,[279] especially if the cat is allowed to go outside. MLV vaccines should be avoided in FeLV-infected cats, if possible, because attenuated agents may regain their pathogenicity in an immunosuppressed animal. FeLV vaccines are not recommended in cats with known progressive or regressive FeLV infections because these vaccines have no effect on the viremia, carrier state or elimination, or clinical FeLV disease in already infected cats.

Treatment of Feline Leukemia Virus-Associated Diseases

In most cases, secondary diseases in FeLV-infected cats are treated in the same way as they are treated in uninfected cats. However, more intensive diagnostic testing and treatment should proceed as soon as an infected cat has been identified. The owner should be forewarned that the response to treatment may take longer than expected. Secondary infectious conditions may require more intensive and prolonged therapy in FeLV-infected cats. FeLV itself does not cause fever, so a search for a concurrent infection must be made in febrile cats. Fevers of unknown origin that are unresponsive to antibiotics may be caused by a co-infecting virus, protozoan, or fungus.

Glucocorticoids and other immune-suppressive drugs should be avoided whenever possible in FeLV-infected cats, unless clearly indicated for a specific problem. These drugs interfere with granulocyte chemotaxis, phagocytosis, and the killing of bacteria, thus compounding the risk of infection.[62] Cats with negative FeLV antigen test results, living in a household with FeLV-shedding cats, should not receive glucocorticoid treatment because it increases the risk of reactivation of a regressive infection. All myelosuppressive drugs should be avoided in FeLV-infected cats because they potentiate the myelosuppressive syndromes caused by FeLV.

Tumors

Although the prognosis is worse when tumors are associated with FeLV,[103,241,430,443] antitumor therapy should be considered in FeLV-infected cats because some patients greatly benefit from it.

Lymphoma and leukemia are usually fatal within 1 to 2 months; however, they can be treated successfully in many cats with chemotherapy, and a few will have remissions that may last several years. Before treatment is considered, a diagnosis of lymphoma must be confirmed by cytology or histology, the condition of the cat should be evaluated to determine its prognosis, and staging of the lymphoma should be assessed. Cats with alimentary lymphoma generally have a poorer prognosis than cats with lymphoma at other sites because anorexia and debilitation are often present. However, cats with a resectable intestinal mass or a mass with well-differentiated histologic features may have extended survival times after treatment. Cats with mediastinal lymphoma have a generally favorable response to chemotherapy.[293,430] Nasal lymphoma seems to remain localized longer than lymphomas in other sites, and radiation in combination with chemotherapy has significantly prolonged survival times. Combinations of chemotherapeutic drugs offer the best chance for complete remission. Single-agent glucocorticoids are minimally effective and should only be considered for palliation if clients have rejected the option of combination chemotherapy. The drugs most frequently administered in combination include cyclophosphamide, vincristine, and prednisone, a protocol called *COP*. Although an old protocol, the *COP* combination is still used frequently and successfully. In comparison with results reported with other combination chemotherapy protocols, the *COP* protocol yields the highest percentage remission and the longest survival rates for cats with lymphoma.[430] The *COP* combination has been effective in achieving complete remission rates of up to 75%.[430] In an older report of 38 cats (of which most cats were FeLV-infected, and the most frequent tumor site was the mediastinum) treated with *COP*, 75% achieved complete remission with a median remission duration of 150 days and a 1-year remission rate of 20%.[57] In a later report, cats from the same geographic area were treated with the same protocol and had a complete remission rate of only 47%, with a median remission duration of 86 days.[314] In this group, few cats were FeLV-infected, and the alimentary form was the most frequent.[314] Therefore, FeLV infection should not prevent lymphoma treatment in a cat.

Other oncologists use the University of Wisconsin–Madison (UWM) doxorubicin-containing multiagent protocol. However, in a study, response rate for 66 cats treated with *COP* was 92% with 73% achieving complete remission, whereas of cats receiving the UWM protocol, only 72% responded, and of these, 64% achieved complete remission. Thus, cats treated with UWM protocol were significantly less likely to respond to treatment than cats treated with the *COP* protocol.[429] Less commonly, L-asparaginase, cytosine arabinoside, and methotrexate are included in FeLV-treatment protocols.

Cats with acute leukemia are difficult to treat because the bone marrow becomes filled with neoplastic blast cells, which must be cleared before the normal hematopoietic precursors can repopulate. This process may take 3 to 4 weeks; therefore, neutropenia and anemia may not be immediately reversible. The remission rate for cats with acute lymphatic leukemia treated initially with vincristine and prednisone is approximately 25%, whereas the rate for cats with AML treated with doxorubicin or cytosine arabinoside) is close to zero.[62] The reason for the extremely poor response may be that a very early stem cell is involved, and nearly total ablation of the bone marrow is necessary to clear the malignant clone.[62] A cat with suspected FeLV-associated chronic lymphocytic leukemia was successfully treated with a combination of prednisone, chlorambucil, cyclophosphamide, doxorubicin, and lomustine.[242]

All these chemotherapeutic drugs are immunosuppressive and some are myelosuppressive, so they can increase the risk of FeLV-associated diseases. Owners must be advised to watch for signs of illness. Infections must be treated quickly and aggressively, especially if they occur at the time of the granulocyte nadir. Although prophylactic antibiotics are not given routinely in the treatment of feline leukemia or lymphoma, broad-spectrum bactericidal antibiotics should be given to FeLV-infected cats, especially if fever or other signs of secondary infection occur. The point at which chemotherapy may safely be discontinued is controversial, but the trend is toward shorter treatment times for cats in continuous complete remission. Previously, most protocols continued for a year or more; now many stop after 6 months of continuous complete remission.

Virally induced feline sarcomas should be treated early, with wide and deep surgical excision. If no metastases are present, but microscopic tumors remain after surgery, radiation can be successful in delaying recurrence. Experimentally FeSV-induced fibrosarcomas in kittens occasionally regressed after treatment with anti-FOCMA serum, but this is unlikely to translate into clinical efficacy.[62]

Hematologic Disorders

Although hematologic disorders are mostly irreversible in FeLV-infected cats, there might a cyclic course and/or some improvement with time. Thus, treatment with blood transfusions (for temporal life support) or bone marrow-stimulation cytokines may be considered.

Anemia can be life-threatening in FeLV-infected cats, and in some cats, blood transfusion is a very important part of the treatment, especially if the anemia is nonregenerative. Most cats respond after the first transfusion. Of 29 anemic (HCT less than 20%) FeLV-infected cats treated with blood transfusions (over 2 weeks), the HCT returned to reference ranges in 8 cats. This may be explained by the cyclic cytopenias that are occasionally seen in FeLV-infected cats. Prednisone may increase the life span of erythrocytes if any component of the anemia is immune mediated, but it should be used only if there is proof of an immune-mediated reaction (e.g., positive Coombs' test result). Occasionally secondary infections (e.g., hemotropic *Mycoplasma* infections) are responsible for the anemia. This type of anemia (which is regenerative) has the best prognosis, and therefore, the possibility of such infectious diseases should always be examined. Deficiencies of iron, folate, or vitamin B_{12} are rare; therefore, replacement therapy is not likely to be helpful.[62] Even though erythropoietin concentrations are often elevated in cats with FeLV-related anemia, treatment with recombinant human erythropoietin (rHuEPO) may be helpful. rHuEPO treatment not only increases erythrocyte counts but also increases platelet and megakaryocyte numbers in animals and humans with clinical disease.[328] In one study, rHuEPO also increased leukocyte counts in cats.[13] No study has been performed involving FeLV-infected cats, but in a study in FIV-infected cats, all treated cats had a gradual increase in erythrocyte counts, hemoglobin concentrations, and HCT and increased leukocyte counts consisting of increased numbers of neutrophils, lymphocytes, or a combination.[13] The recommended dosage is 100 IU/kg given subcutaneously (SC) every 48 hours until the desired HCT (usually 30%) is reached and then as needed to maintain the HCT at 30%. A response may not be seen for 3 to 4 weeks, and if it does not occur, iron supplementation may be required. Iron should not be given to cats that have received transfusions because whole blood contains 0.5 mg/mL of iron, and hemosiderosis may occur in the liver. Anti-erythropoietin antibodies may develop in 25% to 30% of treated animals after 6 to 12 months. Binding of these antibodies to the rHuEPO and the native erythropoietin nullifies their physiologic actions on erythroid progenitor cells, causing bone marrow failure and refractory anemia. However, anti-erythropoietin antibodies dissipate after discontinuation of treatment. Some FeLV-infected cats do not respond to rHuEPO treatment. Reasons for resistance to erythropoietin, other than development of anti-erythropoietin antibodies and iron deficiency, include FeLV infection of bone marrow stromal cells or even concurrent infections with other infectious agents in the bone marrow. In some nonresponsive cats, repeated blood transfusions may be the only treatment possible.

Neutropenia may lead to severe immunosuppression, and antibiotics may be necessary in some cats to prevent secondary bacterial translocation and development of sepsis. Treatment with filgrastim, a granulocyte colony-stimulating factor (G-CSF) that is marketed as recombinant human product (rHuG-CSF) for treatment of neutropenia in humans, has caused transient responses. Filgrastim is used in cats at 5 µg/kg SC every 24 hours for up to 21 days. Potential side effects include bone discomfort, splenomegaly, allergic reactions, and fever.[13,134] Short-term increases in neutrophil counts may be followed by neutropenia with continued use of filgrastim because of development of dose-dependent neutralizing antibodies to this heterologous product after 10 days to 7 weeks. Thus, treatment should not be used for more than 3 weeks.[13,134] Another potential risk is the development of persistent antibodies against endogenous feline G-CSF (at higher dosages), resulting in rebound neutropenia. One study suggests that filgrastim is contraindicated in FIV-infected cats because it led to an increased viral load,[13] but data on the use of filgrastim in FeLV infection are limited. In one study, a small number of naturally FeLV-infected cats were treated with filgrastim; however, treatment did not result in significant changes in neutrophil counts.[239] Other authors reported that it has been used in FeLV-infected cats with cyclic neutropenia with some success.[257] In some FeLV-infected cats with neutropenia, an immune-mediated mechanism is suspected to lead to a maturation arrest in the bone marrow at myelocyte and metamyelocyte stages. Neutrophil counts can be corrected in some of these cats by immune-suppressive doses of glucocorticoids. In animals with myeloid hypoplasia and in the absence of myeloid precursors, direct effects of FeLV are suspected, and glucocorticoids should not be used.

Antiviral and Immunomodulatory Therapy

In many studies, naturally or experimentally FeLV-infected cats have been treated with various substances. More antiviral or immunomodulatory treatment trials are published for FeLV infection than any other infectious disease of cats. Unfortunately, many results are difficult to interpret, and evaluation of data is hampered by the lack of well-controlled clinical trials in which new treatments are compared against a standard care or placebo. No treatment has been proved effective in clearing FeLV infection. To be effective, an agent must inhibit viral replication and allow for recovery of the immune system. Lifelong treatment may be required; thus, the agent should be effective when given orally and should be relatively nontoxic and inexpensive. No such agent has been found to treat cats with FeLV infection (see Chapter 2).[162]

Antiviral Chemotherapy

Most antivirals are human drugs, specifically intended for treatment of HIV infection, but some drugs are active against FeLV in vitro. Some of the anti-HIV drugs have been used to treat experimentally and naturally FeLV-infected cats, and improvement of clinical signs and prolongation of life can be achieved in some cats using antiviral therapy (see Chapter 2).

Zidovudine (3′-azido-2′,3′-dideoxythymidine [AZT]) (see Chapter 2) is effective against FeLV in vitro and has been used in experimental and field trials in FeLV-infected cats.[428] See Zidovudine, Chapter 2, and the Drug Formulary in the Appendix, for specific information on its use in treatment of cats infected with FeLV.[158,159,166]

Didanosine (ddI) is also used to treat HIV infection and has been used successfully in experimentally FIV-infected cats (see Chapter 2). The drug inhibits FeLV replication in vitro[428]; however, controlled in vivo studies confirming the efficacy of ddI in cats with FeLV infection are not available.

Zalcitabine (ddC) also is currently used to treat HIV infection (see Chapter 2). It is effective against FeLV in vitro[197,348,428] and has been used in experimental studies to treat FeLV-infected cats.

Suramin is one of the oldest known antimicrobial agents. It has been used against parasitic diseases but also has some antiviral activity. It was used to treat FeLV-infected cats, although only a limited number of cats have been evaluated (see Chapter 2).

Foscarnet is a pyrophosphate that inhibits nucleic acid synthesis (see Chapter 2). It has in vitro activity against FeLV,[421] but no reliable data exist on its efficacy in cats in vivo. Toxicity limits its use, as it causes nephrotoxicity and myelosuppression in cats (see Drug Formulary in the Appendix).

Ribavirin (RTCA) is a broad-spectrum triazole nucleoside that is active against a variety of DNA and RNA viruses (see Chapter 2). RTCA is active against FeLV in vitro,[135] but it is of limited usefulness

because cats are extremely sensitive to its toxicity (see the Drug Formulary in the Appendix).

Antibody Therapy

Antibody therapy has been used in an attempt to treat FeLV. Antibodies were derived from immune cats or were obtained as murine MABs to epitopes of gp70. Antibodies have successfully treated experimentally infected cats, but only when given within 3 weeks of infection. Naturally infected cats showed no response, even though the MABs persisted longer in viremic cats than in normal control animals. FeLV-infected cats treated with antibodies developed residual CICs[62] that could potentially cause adverse reactions.

Immunomodulatory Therapy

Immune modulators or cytokine inducers have been used in the treatment of FeLV-infected cats. Attempts to stimulate the immune response have been used more extensively in FeLV infection than in any other infectious disease in veterinary medicine. However, controlled studies including large numbers of naturally infected cats do not exist for most of these agents.

Human IFN-α has immunomodulatory and antiviral activity (see Chapter 2 and the Drug Formulary in the Appendix). FeLV replication is inhibited in vitro by human IFN-α, and several studies have been performed on the use of human IFN-α in FeLV-infected cats. To evaluate the direct effect on infected cells, human IFN-α and feline IFN-ω were added to a chronically FeLV-infected FL74 cell line. IFNs did not apparently affect viral protein expression; however, RT activity, directly proportional to the amount of infectious free virions, decreased with increasing concentrations of IFNs and longer treatment times. In addition, the IFNs decreased viability and increased apoptosis of FeLV-infected cells, but not of noninfected cells.[56] Two treatment regimens of recombinant human IFN-α have been used in cats: high-dosage SC injection (10^4 to 10^6 IU/kg every 24 hours) or low-dosage oral administration (1 to 50 IU/kg every 24 hours). Parenteral (SC) administration of IFN-α leads to the development of neutralizing antibodies that inactivate the drug. With oral use, antiviral effects are unlikely, but immunomodulatory activity may be present. Given orally, IFN-α is inactivated by gastric acid and, like other proteins, destroyed by trypsin and other proteolytic enzymes in the duodenum; therefore, it is not absorbed and cannot be detected in the blood after oral administration.[43] However, IFN-α may bind to mucosal receptors in the oral cavity, stimulating the local lymphoid tissue and leading to cytokine release on lymphatic cells in the oral or pharyngeal area, triggering a cascade of immunologic responses that finally act systemically.[236] Thus, the rational behind the use of oral low doses is to mimic natural defense processes, and when comparing low-dose oral IFN-α with higher oral doses, higher dose did not improve the effect.[68] Treatment of experimentally infected cats with high-dose human IFN-α (1.6×10^4 IU/kg to 1.6×10^6 IU/kg SC) either alone or in combination with AZT resulted in significant decreases in circulating FeLV p27 antigen. However, cats became refractory to therapy 3 or 7 weeks after the beginning of treatment because of anti-IFN-α antibody development.[467] In naturally FeLV-infected cats using a similar high-dose treatment regimen, however, treatment with human IFN-α (1×10^5 IU/kg SC every 24 hours for 6 weeks) with or without AZT did not lead to a statistically significant improvement of clinical, laboratory, immunologic, or virologic parameters.[160] Low-dose oral IFN-α was used in a placebo-controlled study in experimentally induced FeLV infection; 0.5 IU/cat (8 cats) or 5 IU/cat (5 cats) were treated orally (after experimental challenge) on 7 consecutive days on alternate weeks for a period of 1 month.[69] No difference was found in the development of viremia between groups; however, treated cats had significantly fewer clinical signs and longer survival times when compared with the placebo group (with a better response in the cats given 0.5 IU/cat). Several uncontrolled studies reported a beneficial response in field cats when treated with low-dose oral IFN,[414,435,457] but they include only a limited number of cats and are difficult to interpret without control groups. In a larger study, outcome of 69 FeLV-infected cats with clinical signs that were treated with low-dose oral IFN (30 IU/kg for 7 consecutive days on a 1-week-on, 1-week-off schedule) was compared with historical controls, and significant longer survival times were reported in the treated cats.[457] In a placebo-controlled study, treatment of ill client-owned FeLV-infected cats with low-dose oral IFN-α (30 IU/cat for 7 consecutive days on a 1-week-on, 1-week-off schedule), either alone or in combination with *Staphylococcus* protein A (SPA), did not result in any statistically significant difference in FeLV status, survival time, clinical or hematologic parameters, or subjective improvement in the owners' impression when compared to a placebo group.[303] Thus, this controlled study was not able to demonstrate efficacy.

Feline IFN-ω has been licensed for use in veterinary medicine in some European countries and Japan (see Chapter 2 and the Drug Formulary in the Appendix). Cats will not develop antibodies to IFN-ω because of its homologous origin. Long-term parenteral use is possible. Feline IFN-ω inhibits FeLV replication in vitro[372] by decreasing viability and increased apoptosis of FeLV-infected cells.[56] In a placebo-controlled field study in France, 48 cats with FeLV infection were treated with IFN-ω at 1×10^6 IU/kg every 24 hours SC on 5 consecutive days in three 5-day series beginning on days 0, 14, and 60. Supportive therapies were used in both groups, and cats were monitored for up to 1 year. There was a statistically significant difference in the survival time of treated versus untreated cat after 9 months, but not after 1 year.[80] No virologic parameters were measured throughout the study to support the hypothesis that IFN-ω actually had an anti-FeLV effect rather than inhibiting secondary infections. Thus, additional studies are needed. A treatment protocol of 1×10^6 IU/kg IFN-ω every 24 hours SC on 5 consecutive days has been suggested[80] but may be modified in the future. No side effects have been reported in cats.

Staphylococcal protein A (SPA) is a bacterial polypeptide product purified from cell walls of *Staphylococcus aureus* Cowan I that has been used in various modalities to treat FeLV-infected cats (see Chapter 2 and the Drug Formulary in the Appendix). Interest was first generated when plasma from FeLV-infected lymphoma-bearing cats was passed over SPA or *S. aureus* columns to remove CICs and then returned to the cats. Many cats were treated in this manner, and in some reports, a high rate of tumor remission and conversion to FeLV-negative status was observed; in others, responses were less dramatic and short-lived. Subsequently, it was determined that SPA and other products may have leached from the filters and columns used for immunosorption and been returned to the cats as contaminants in the treated plasma.[156] The possibility that these products exerted a positive immunomodulatory effect caused investigators to treat cats with small doses of SPA. In such a study including kittens with experimental FeLV infection, treatment with SPA (7.3 mg/kg intraperitoneally [IP] twice weekly for 8 weeks) did not correct anemia or improve humoral immune function.[303] In an experimental study involving 17 cats (5 FeLV-infected viremic cats, 6 FeLV-infected nonviremic cats, and 6 uninfected controls), no difference was seen in viremia and immune response, but a stimulation of bone marrow granulocytic lineage could be detected.[246] In a placebo-controlled field study, treatment of ill client-owned FeLV-infected cats with SPA (10 mg/kg, IP, twice per week for up to 10 weeks) did not cause a statistically significant difference in the FeLV status, survival time, or clinical and hematologic parameters when compared to a placebo group, but it caused a significant improvement in the owners'

subjective impressions on the health of their cats. Interestingly, when SPA was combined with low-dose (30 U/day) oral IFN-α on alternate weekly intervals, the effect was less than with SPA alone.[303]

Propionibacterium acnes, formerly *Corynebacterium parvum*, consists of a killed bacterial product that stimulates macrophages resulting in release of various cytokines (see Chapter 2 and the Drug Formulary in the Appendix). It is available for veterinary use and has been used in treating FeLV-infected cats, but no prospective studies have been performed. Veterinarians have described their clinical experience in round-table discussions and anecdotal reports,[257,265] but its efficacy has yet to be evaluated in controlled studies.

Bacille Calmette-Guérin is a cell wall extract of a nonpathogenic strain of *Mycobacterium bovis* that has immunomodulatory effects (see Chapter 2) and has been used to treat kittens experimentally infected with FeSV. However, bacille Calmette-Guérin was not able to prevent tumor development or increase the survival rate.[19]

Biological extract of Serratia marcescens is commercially available and stimulates normal feline macrophages derived from bone marrow to release IFNs, leading to elevation in body temperature and neutrophil count (see Chapter 2). In a study with experimentally FeLV-infected cats, weekly treatment with biological extract of *S. marcescens* failed to prevent or reverse viremia in cats when initiated before or 6 weeks after inoculation with FeLV.[94]

Parapox virus avis and parapox virus ovis are attenuated poxviruses that induce IFN and colony-stimulating factors and activate natural killer cells (see Chapter 2). Initial reports suggested that these compounds were able to cure 80% to 100% of FeLV-infected cats.[198] However, two placebo-controlled double-blind trials using the same treatment protocol were not able to repeat these striking results.[163,164] More than 20 immunologic, clinical, laboratory, and virologic parameters were examined (including FeLV p27 antigen concentration, clinical signs, lymphocyte subsets, and survival time), but no statistically significant differences could be demonstrated between paramunity inducer and placebo administration.[32,163,164]

Acemannan is a water-soluble, long-chain complex carbohydrate (mannan) polymer derived from the aloe vera plant that has immunomodulatory activity (see Chapter 2 and the Drug Formulary in the Appendix). In one noncontrolled open-label trial, 50 cats with natural FeLV infection were treated with acemannan (2 mg/kg, IP, every 7 days for 6 weeks). At the end of the 12-week study, 71% of the cats were known to be alive.[394] All cats remained FeLV-antigen positive, and no significant change was detected in clinical signs or hematologic parameters. The fact that the study did not include a control group and clinical and laboratory evaluations failed to document improvement from pretreatment evaluations makes it difficult to determine whether the use of acemannan improved the outcome of infection.

Levamisole (see Chapter 2) is a broad-spectrum anthelmintic with immunomodulatory activity that has been given to FeLV-infected cats,[59] but its effect has never been substantiated by controlled studies. Levamisole remains an investigational therapy.

Diethylcarbamazine (DEC) is another antiparasitic and immunomodulatory agent widely used (see Chapter 2), that may mitigate the course of FeLV infection in cats. Uncontrolled studies have suggested that continuous oral DEC treatment given shortly after evidence of FeLV infection may prevent or delay FeLV-associated lymphopenia and prolong survival.[234,235] In one controlled study, its therapeutic effect against FeLV infection was investigated in 24 specific-pathogen free kittens experimentally infected with a lymphoma-causing strain of FeLV. The kittens were divided into four groups and received a high dosage of DEC (12 mg/kg every 24 hours), a low dosage of DEC (3 mg/kg every 24 hours), AZT (15 mg/kg every 12 hours), or a placebo orally for 10 weeks. Although AZT was effective in preventing progressive infection, neither dosage of DEC was effective. However, AZT as well as both dosage levels of DEC prevented lymphoma development.[324] DEC can cause severe side effects, mainly including hepatic injury.[39]

PREVENTION

Preventative methods, including general precautions, testing and removal strategies, and vaccination, have been very successful in significantly decreasing the prevalence of FeLV infection.

General Prevention of Infection

FeLV is prevalent in body excretions (highest concentrations in saliva) in cats with progressive infection that pose an immediate risk to other cats in their environment. Because of its environmental lability, direct contact among cats and immediate fomite transfer are the major risk factors. Progressively infected cats should be physically separated from other cats in the environment. In a veterinary hospital, FeLV-shedding cats can be housed in the same ward with other hospitalized patients as long as they are housed in separate cages and certain precautionary measures are taken. FeLV is very fragile, surviving only minutes at most outside the host animal. The virus is susceptible to all disinfectants including common soap; thus, simple precautions (e.g., hand washing) and routine cleaning procedures can prevent transmission in the hospital setting. FeLV-infected patients should be housed in individual cages and confined to them throughout their hospitalization. They should never be placed in a "contagious ward" with cats that have other infections such as viral respiratory disease. Animal caretakers and other hospital staff members should be advised to wash their hands between direct contacts with patients (primarily to protect the FeLV-infected, immune-suppressed cat) and after cleaning cages and litter boxes. Dental and surgical instruments, endotracheal tubes, and other items potentially contaminated with body fluids of a FeLV-infected cat should be thoroughly cleaned and sterilized between uses. Fluid lines, multidose medication containers, and food can become contaminated with body fluids (especially blood or saliva) and should not be shared among patients. FeLV can be transmitted hematogenously; therefore, all feline blood donors should be screened and confirmed to be free of infection before donating blood.[255,260] FeLV was detected in the corneal tissues of cats by PCR and immunohistochemistry[179]; therefore, screening potential corneal donors for FeLV infection is also generally warranted.

Test and Removal Strategy

When FeLV was first described in the mid-1960s, the highest rate of infection was found in large multicat households and catteries. In contrast, free-roaming cats had lower rates of infection, and those housed in single-cat households were only rarely infected. Convenient and reliable testing became available in the mid-1970s. Very quickly, cat breeders implemented test and removal programs, which proved to be extremely effective for eliminating the virus from catteries. The most dramatic example was a mandatory test and removal program in the Netherlands in 1974 that was imposed on all cat breeders.[453] When testing was first implemented, the prevalence of FeLV in purebred catteries was 11%. Within 4 years, the rate was reduced to less than 2%, and no infected cat has been reported since 1984. FeLV should be considered an abnormality in a well-run cattery. Many stray-cat shelters also implement testing in their conditioning protocols, thus further reducing the rate of FeLV infection.[257] Epidemiologic studies suggest that the testing and removal strategies have more influence than vaccination on the decrease in prevalence.[380]

Vaccination

Because most naturally exposed cats produce antibodies to virus and become immune, it is theoretically possible to produce an effective

vaccine. However, accomplishing the task proved to be more difficult than anticipated. The mechanism of protection against FeLV and the role of neutralizing antibodies and of CMI are not completely understood.

Vaccine Development and Efficacy

Development of a safe and effective vaccine against FeLV presents a challenge that other infections do not. Early vaccines carried a higher risk of anaphylaxis than did other feline vaccines. Original prototypes of inactivated virus vaccines not only were ineffective but also increased the severity of the immunosuppression. Live virus vaccines produced immunity, but some vaccinated kittens developed clinical disease from "attenuated" virus. Researchers were also concerned that the vaccine virus could integrate into the host genome and later cause FeLV antigen-negative lymphomas; thus, most vaccine research focused on the use of whole killed virus preparations or subunit vaccines.

The first anti-FeLV vaccine was licensed in 1985. Since that time, this original vaccine has undergone modifications, and several other products have appeared on the market. Licensed vaccines use whole killed virus, and most contain adjuvant or genetically engineered recombinant parts of the virus. Vaccination does not interfere with testing for FeLV, unless blood for FeLV testing is taken immediately after receiving the vaccine.[260] Recommendations for most vaccines are for two SC doses for initial protection followed by booster vaccinations; some vaccines are licensed for yearly boosters, some for up to 3-year boosters, and the ones with longer booster intervals are generally preferred (see Chapter 100 for additional information on FeLV vaccination recommendations).[219a,366] It is not necessary to administer vaccines from the same manufacturer for boosters.[222] Not all cats respond equally to FeLV vaccination, and immunosuppressed cats may fail to develop immunity. The results of a 5-year field study to control FeLV infection by vaccination in a colony of 30 domestic adult cats naturally exposed to infection suggested that the vaccination was effective in FIV-negative cats, but failed to protect FIV-infected cats against FeLV.[20]

The relative efficacy of the vaccines is the subject of much controversy. Many of the published vaccine efficacy trials were performed or funded by the manufacturers, without having simultaneous evaluation of more than one vaccine. Furthermore, testing protocols vary widely among studies, making meaningful comparison difficult. Because of the natural resistance of cats (especially older cats) to FeLV infection, investigators often use artificial immunosuppression (e.g., glucocorticoids) and administration of large viral doses to increase the challenge virulence in FeLV vaccine studies. Some use immunosuppressed vaccinated and control cats before intranasal challenge with virulent virus, and others have performed parenteral challenge with large doses of virus without immunosuppression. The relationship of these challenges to natural exposure has been questioned,[53,181,191,253,254] making it difficult to know what the vaccine's actual effect in a natural exposure environment would be. Some studies have involved natural challenges when vaccinated and control cats lived together with FeLV-shedding cats.[137,245] This type of challenge is preferable because it is more comparable to the natural situation, but no standard challenge protocol has been accepted by vaccine manufacturers. This type of natural exposure challenge experiment, in which naïve cats are housed together with a FeLV-shedding cat (which is comparable to natural multiple-cat household situations), provides an environment with a very high infection pressure. In this high-pressure situation, none of the licensed vaccines showed enough efficacy. Therefore, it is not safe to bring a FeLV-shedding cat into a household with FeLV-negative cats, even if these cats are vaccinated. Furthermore, so far most FeLV vaccine studies were conducted assaying parameters, such as virus isolation and antigen detection. Using real-time PCR, cats initially believed to be immune to FeLV infection, with negative results on ELISA testing, were found to be infected with provirus after virus exposure. Thus FeLV vaccines that protect cats from antigenemia were found not to prevent proviral integration and minimal viral replication after challenge. Nonetheless, vaccines protected cats from FeLV-associated disease and prolonged life expectancy,[186] but FeLV provirus was found to persist for years even in vaccinated cats, and recurrence of viremia and disease development was observed in some cats.[187] One independent study, using a simultaneous challenge model, has been conducted comparing the response to IP challenge of cats, vaccinated 4 months earlier, with each of the USDA-licensed commercially available FeLV vaccines.[438] Whole-cell inactivated and adjuvanted vaccines completely protected the cats against postchallenge viremia as detected by both antigen and nucleic acid methods, whereas the inactivated subunit and nonadjuvanted vaccines offered partial protection.

Short of challenge with virulent virus, no accurate postvaccination measures exist that can determine whether cats are protected after vaccination. Neutralizing antibody titers develop in only a few vaccinated cats despite their being protected against challenge infection,[210] and many properly vaccinated and protected cats in the field and in challenge studies do not have detectable antibodies.[97,438] The immune mechanism protecting cats from persistent viremia is CMI through the effects of CTLs.[110,143] Although neutralizing antibody titers do not predict postvaccinal protection, they may indicate which cats are protected after recovery from natural infection. Presence of neutralizing antibodies in cats clearly correlates with resistance to subsequent infection, and passive transfer of antibodies can protect cats. In a study in which cats were immunized with the transmembrane envelope protein p15E of FeLV, high titers of neutralizing antibodies specific for FeLV were induced. In contrast, sera from progressively FeLV-infected animals failed to recognize the neutralization-relevant epitopes in p15E.[248] Although ELISA and other antibody assays against different envelope antigens exist, only neutralizing antibody titers are predictive of protection. Unfortunately, testing for neutralizing antibody is not commercially available.

Injection Site–Associated Sarcomas

A clear epidemiologic association exists between FeLV (and rabies) vaccinations and later development of soft-tissue sarcomas at the injection site (sarcomas referred to as *injection site sarcomas* [ISSs], *feline injection site-associated sarcomas*, *vaccine site-associated sarcomas*, and *vaccine-associated feline sarcomas*)* (see also Chapter 100). The most frequently occurring type of these soft-tissue sarcomas is fibrosarcomas, but undifferentiated sarcomas, rhabdomyosarcomas, chondrosarcomas, osteosarcomas, and malignant fibrous histiocytomas are also found. The estimated incidence ranges from 1 tumor per 1000 vaccines to 1 tumor per 10,000 vaccines.[286] Reported rates of reactions were 0.32 vaccine-associated sarcomas per 10,000 vaccines and 11.80 postvaccinal inflammation reactions per 10,000 vaccines in cats.[128] If inflammatory reactions are a necessary prelude to sarcoma, then these rates suggest that 1 in 35 to 40 reportable inflammatory reactions transitions to sarcoma. These tumors may occur as soon as 4 months or as long as 2 years after vaccination, with a median of approximately 1 year.[225] The tumors are derived from the granulomatous inflammation at the injection site. In addition to FeLV and rabies vaccination, other vaccines[79] and potentially every other SC, intradermal, or intramuscular injection (e.g., certain long-acting injectable medications)[226] and other irritations (e.g., chips, bite wounds) can

*References 77, 176, 177, 225, 226, 286.

cause these tumors in cats (although this is much less likely). In addition, a genetic predisposition of the individual cat seems to play a role. A case-control study (50 domestic shorthaired cats with a confirmed histopathologic diagnosis of ISS and 100 disease-free matched controls) investigated the association of polymorphisms in the genomic sequence of the feline p53 gene with a predisposition to ISSs, and a strong association was found between ISS and the presence of specific nucleotides at two of the polymorphic sites, with the strongest association for a single-base insertion in intron 7.[21] However, the apparent association between inflammation or injury at an injection site and the risk of tumor development cannot be absolutely confirmed. Cats seem to have an unusual response to adjuvants, often added to inactivated vaccines.[45] However, these tumors have also been reported in ferrets[319] and very occasionally in dogs.[446] Vaccine-associated inflammation is thought to promote malignant transformation. Traces of adjuvants can be seen in the inflammatory reaction and later in histologic sections of tumors in the transformed fibroblast.[176] Intracellular crystalline particulate material was found in an ultrastructural study in 5 of 20 investigated tumors, and in one case was identified as aluminum based.[289] Although no specific vaccine or adjuvant has been incriminated,[226] local irritation from adjuvants might stimulate fibroblasts to the point that malignant transformation occurs.[253] FeLV and FeSV themselves are not involved in the tumor development.[94] Also, replication or expression of endogenous retroviruses is obviously not involved.[229]

The recognition of the malignancies associated with widespread annual vaccination has led the AAFP to revise vaccination recommendation for cats[366] and to create new guidelines for testing of feline retrovirus infections.[260] Care should be taken in every cat to weigh the risk of FeLV infection against the risk of receiving the vaccine. The AAFP questions the automatic annual revaccination in cats and recommends tailoring vaccine protocols for each feline patient.[366] Only cats at risk of contracting the infection should be vaccinated.[232] Cats living in closed households have no risk to acquire FeLV infection and should not be vaccinated. New cats in multicat households or in shelters should be tested before being introduced. Testing by ELISA-based methods is recommended before FeLV vaccination so that only cats having positive results for FeLV antigen undergo vaccination. FeLV vaccination of cats having positive results for FeLV antigen is not beneficial, although it does not lead to progression of FeLV infection. The absence of existing antigenemia as measured by ELISA-based methods is the best predictor as to whether the cat may benefit from the vaccine. PCR testing is not recommended before vaccination, in an attempt to identify provirus or virus in latently infected cats. Adult cats have an age resistance, and in an older cat the risk of developing a vaccine-associated sarcoma is most likely higher than the risk of developing a persistent FeLV viremia. Unfortunately, no studies provide data on the age at which vaccination should be stopped.

The AAFP recommends administering any vaccine with a FeLV component SC in the left rear leg, whereas the rabies vaccination is given in the right rear leg ("left for leukemia, right for rabies") as far distally as possible.[366] Intramuscular injection of vaccines is contraindicated because the tumors develop with the same frequency but are more difficult to detect early. Any nodule that is present longer than 3 months at an injection site should be removed and examined histologically. Injecting distally in the leg aids in the treatment of subsequent sarcomas (by amputation of the leg) because these tumors are very difficult to completely excise and often recur after resection.[286] Administration of the vaccine between the scapulae is contraindicated because tumor resection is almost impossible in this location. To assess the acceptance of the AAFP recommendation, a study including 392 cats with ISS compared anatomic location of tumors between ISSs diagnosed before and after publication of the Vaccine-Associated Feline Sarcoma Task Force vaccination recommendations published in 1999.[11] From before to after publication of the vaccination recommendations, proportions of ISSs significantly decreased in the interscapular (53% to 40%) and right and left thoracic (10% to 4% and 9% to 1%, respectively) regions, whereas proportions of ISSs significantly increased in the right thoracic limb (1% to 10%) and the combined regions of the right pelvic limb with right lateral aspect of the abdomen (13% to 25%) and the left pelvic limb with left lateral aspect of the abdomen (11% to 14%). Thus, despite publication of the vaccination recommendations, a high proportion of tumors still developed in the interscapular region. There was also an increase in lateral abdominal ISSs, which are more difficult to treat and are likely attributable to aberrant placement of injections intended for the pelvic limbs. Thus, veterinarians are complying with vaccination recommendations to some extent, but they need to focus on administering vaccines as distally as possible on a limb to allow for complete surgical margins if amputation of a limb is required.[393] FeLV vaccines that are licensed for 3-year boosters should be used if available. Vaccines that do not contain adjuvant should be used instead of adjuvant-containing vaccines. A licensed recombinant vaccine containing FeLV genes was cloned in a canarypox vector so that the genetic information of the FeLV proteins is integrated into the canarypox genome with which it enters the cell. Thus, the FeLV proteins are produced in the cat, leading to antibody production and response of cellular immunity. Safety of this type of vaccine has been proven, and good immediate and long-term efficacy in this vaccine has been established.[352,427] In a natural challenge experiment, vaccinated cats were housed together with FeLV-shedding cats for 27 weeks; the canarypox vaccine was as effective as other commercially available vaccines.[137] This vaccine also can be administered by a needle-less air injection system. The advantage of the canarypox technology is that it does not need inflammation at the injection site because it is distributed in the body by the canarypox virus and exposed to the immune system by other mechanisms. In one study in rats, inflammation at the injection site was less with recombinant canarypox vaccines compared to conventional vaccines.[287,288] In a study in cats, the typical granulomatous inflammation did not develop at the injection site when using this type of vaccine.[24] A study investigating the subcutaneous tissue response to administration of a single dose of multicomponent vaccine in the cat reported similar findings. Three groups of 15 cats were injected with one of three vaccine products and saline was used as a negative control; cats in group A received nonadjuvanted vaccine; cats in group B received vaccine with a lipid-based adjuvant; and cats in group C were vaccinated with a product adjuvanted with an alum-Quil A mixture. On days 7, 21, and 62 postvaccination, there was significantly less inflammation associated with administration of nonadjuvanted vaccine, and cats receiving adjuvanted vaccines had evidence of residual adjuvant material accumulated within macrophages seen at 62 days postvaccination.[74] A DNA vaccine has also been developed for FeLV that contains all FeLV genes and the feline IL-18 gene as an adjuvant. It has been highly protective in challenge experiments with kittens by producing FeLV-specific CTLs and protection against challenge infection,[110,143,211] but this type of vaccine is not yet available. For further information on vaccination for FeLV infection, see Chapter 100 and Web Appendix 2.

PUBLIC HEALTH CONSIDERATIONS

Because FeLV is known to be contagious, concern arose about the possible danger of FeLV to humans. Numerous facts suggest that human infection is not impossible. The virus does grow in human bone marrow cells in culture.[315] Lymphoma has been experimentally

induced by injection of large doses of virus into neonatal pups and marmosets.[367] One epidemiologic study linked prior contact with sick cats to subsequent development of childhood leukemia. The contact between FeLV-infected cats and children with leukemia was double that of contact between healthy children and healthy cats.[36,342] Cell-bound antibody believed to be directed toward FeLV RT has been found on malignant cells of humans with chronic myelocytic leukemia in blast cell crisis. Veterinarians were shown to have a higher death rate from leukemia than a control population of physicians and dentists.[30,53,58,106,140] However, the increased death rate could also be explained by their higher exposure rate to radiation.

Epidemiologic studies searching for FeLV or antibodies to any of its components in humans have been confusing and inconclusive. Some investigators have found antibodies to FeLV in human beings with leukemia and owners of viremic cats,[41,107,208,331] whereas others using more specific radioimmunoassays have obtained negative results. No human being has ever been found to be viremic with FeLV. PCR was used without success to find FeLV sequences in blood and bone marrow of young and adult humans with leukemia.[326] No case of human leukemia has ever been traced to FeLV. One explanation for the discrepancy between culture of the virus in human cells and the absence of proof of human infection may be related to the lytic action of human complement on the virus. Although it is almost impossible to prove a negative hypothesis, it appears that FeLV is not a human health hazard. A potential risk to immunosuppressed people who live in close contact with FeLV-infected cats cannot be completely excluded and should be discussed with such owners. However, the risk involved is mainly from secondary zoonotic infections that an immunosuppressed cat might acquire and potentially transmit to an immunosuppressed human.

CHAPTER 12

Feline Immunodeficiency Virus Infection

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■ References ■ Images ■ Web Tables, Boxes, and Appendices

ETIOLOGY

Feline immunodeficiency virus (FIV), a lentivirus that shares many properties characteristic of other lentiviruses such as human immunodeficiency virus (HIV), retains great interest as a model of lentiviral pathogenesis and prevention. Given the enormous volume of literature generated since the first description of FIV in 1986, the principal goal of this chapter is to familiarize the reader with current, clinically important concepts. Readers interested in a review of the genetic organization, biology, and life cycle of FIV, details of FIV gene function and gene products, and comparisons to other lentiviruses are referred to other sources.*

Although discussion of the FIV genome and its products is not the focus of this chapter, several FIV genes have clinically important aspects. Regions in the viral integrase enzyme determine the site of binding and integration of FIV provirus into the host cell DNA, which can influence host cell function.[927,928] The envelope (*env*) gene and its proteins[768] also are of clinical importance. Field isolates of FIV are divided into several subtypes (clades) based, in part, on sequence differences in a hypervariable region of the *env* gene.[766,1056] Within regions, numerous subtypes exist, owing to a high level of genetic recombination, especially of the *env* gene. Worldwide, five major subtypes have been recognized: subtypes A, B, C, D, and E. Additionally, new subtypes have been described in cats from Texas (subtype F), New Zealand, and Portugal.[251,394,728,779,1071] Available studies suggest that subtypes A and B predominate in the United States and Canada, with some cats infected with subtypes C and F.[37,78,850,1070a,1099] These studies also suggest regional differences in subtype distribution.[1070a] In Australia, the presence of subtypes A and B has been described,[467,505] and in New Zealand, subtypes A and C.[393a,394,503,506] In Africa, subtype A and in South America, subtypes B and E have been found.[155,654,1000] Subtypes B, C, and D predominate in Japan and other Asian countries, although subtypes A and E have also been observed.[413,542,722,728,1032] European cats are infected with subtypes A, B, C, and D, with subtype A being the major subtype in the northern countries (e.g., Germany, The Netherlands), and subtype B being more important in southern countries (e.g., Italy).[250,803,980,979] Analysis of European FIV subtypes has suggested that subgroupings within a given subtype are also possible, reflecting the genetic plasticity of FIV.[980] Differences in *env* antigenic determinants presents potential obstacles in the development of FIV vaccines protective against widely prevalent, and different, isolates of FIV[409,936,939] (see Prevention). Naturally infected cats can harbor multiple subtypes,[545,748] and superinfection indicates a lack of cross-protection between subtypes.[151,545,748] Evidence suggests that exchange of gene segments encoding the *env* protein from different subtypes can occur between isolates in superinfected cats.[151] Such

*References 11, 12, 25, 66, 71, 80, 119, 120, 142, 147, 157–159, 218, 231, 257, 265, 267, 268, 278, 326, 328, 333, 334, 338, 340, 377, 458, 461, 462, 514, 515, 551, 552, 564, 597, 606, 618, 620, 630, 631, 651, 652, 683, 709, 710, 714, 717, 724, 736, 757, 776, 807, 837, 888, 897, 917, 919, 926, 966, 988, 990, 996, 1015, 1038, 1076, 1098, 1102, 1113, 1114, 1117, 1136.

recombination events could be one factor in the emergence of new subtypes.

Env properties are also clinically relevant because they determine cell tropism* and influence pathogenicity.[480,484,531,766,1056] Interactions of FIV *env* proteins with host cell are critical initial steps during host cell infection, making them potential targets for therapeutic intervention.[426,691,1080] *Env* proteins are targets of immune responses,† and differences in, or conservation of, *env* sequences may reflect selection pressures exerted by the immune response of the infected cat. FIV *env* sequences evolve through acquisition of mutations during the course of infection, potentially contributing to variants that resist neutralization or contribute to disease progression.[538]

EPIDEMIOLOGY

Prevalence

FIV is common worldwide, and its prevalence varies among geographic locations. Across the United States and Canada, the reported antibody prevalence of FIV ranges from approximately 4% to 24%[587,613,614,629,847a]; the largest North American study of more than 18,000 cats identified an overall antibody prevalence of 2.5% with an 18.2% antibody prevalence in sick cats that were tested.[594] In this study, the antibody prevalence in western-region cats was lower than those in other regions, in contrast to older studies of fewer animals that documented little in the way of regional differences.[167,567] In Europe, antibody prevalence is highly variable. Some countries (e.g., northern European countries)[352] report few infected cats, and others, such as Italy, with large populations of free-roaming cats have prevalence rates that can approach 30%.‡ For similar reasons, Japan also has very high infection rates.[721] Pockets of high prevalence in countries with low overall infection rates, such as 47% reported in one group of feral cats in the United Kingdom, can also be seen,[151] likely reflecting local cat population dynamics.[29]

Within a given population, the prevalence of FIV in healthy cats is usually lower than in sick cats.§ Antibody prevalence in virtually all surveys is higher in male cats than it is in female cats, which is considered the result of higher rates of virus transmission among biting and fighting cats.‖ Similarly, the risk of infection is higher in cats that spend more time outdoors.[352,594] Adult cats are infected more often than adolescent cats and kittens are,[42,352,594,712] which again likely reflects aggressive behavior between cats as the predominant means of natural transmission. In antibody surveys, it is uncommon for enzyme-linked immunosorbent assay (ELISA)-positive results to be confirmed by other methods; thus, true infection prevalence may be overestimated, especially in the healthy cat population, because of false-positive results or potentially vaccinated cats.

Evidence from retrospective antibody surveys suggests that FIV has been present in the domestic feline population since at least 1966.[921] Infection with lentiviruses related to FIV has been reported in Florida panthers (*Puma concolor coryi*) and many other nondomestic feline species in United States zoos, as well as in free-roaming nondomestic felids in the United States, Europe, Africa, Saudi Arabia, and Asia.¶ Interestingly, presence of antibodies against FIV has been reported in spotted hyenas (*Crocuta crocuta*),[375] but whether this observation reflects cross-reactivity with a novel hyena lentivirus or reactions arising from their exposure to lion (*Panthera leo*) lentiviruses is not known. The greater diversity of viral nucleic acid sequences and the decreased pathogenicity of the nondomestic felid isolates, compared with those that affect domestic cats suggest that nondomestic felids have been living with the virus longer and that the domestic cat strains may have emerged from nondomestic strains.[129,750] Readers interested in learning more about lentiviral infections in nondomestic cats are referred to other sources (Web Table 12-1).

Domestic cats are susceptible to persistent infection with isolates from nondomestic felids, but the clinical and immunologic abnormalities that develop after infection with domestic cat isolates are typically not observed.[1004,1051] Furthermore, cross-infection studies suggest that infection with a nondomestic feline lentivirus (lion or puma lentivirus) may blunt the immunologic and virologic responses to subsequent FIV infection.[1048,1049] Probable transmission of an FIV isolate from domestic cats to an exotic cat species has also been documented.[729]

Transmission

In natural settings, FIV is transmitted primarily by parenteral inoculation of virus present in saliva or blood, presumably by bite and fight wounds, accounting for the higher prevalence in male cats. Evidence supporting the importance of this route of transmission is the observation that FIV can be found in salivary gland epithelium[662,772] during acute infection, as well as in saliva, blood lymphocytes, and plasma or serum.[662] Experimentally induced bites can transmit virus from infected to naïve cats. Experimentally, FIV is easily transmitted by all parenteral routes (intravenous, subcutaneous, intramuscular, intraperitoneal) using cell-free or cell-associated virus.

In experimental settings, high rates (over 50%) of transmission in utero and postparturition via milk have been documented in queens with acute and chronic FIV infections.* The presence of higher viral loads in milk than in milk-secreting cells or plasma suggests that virus is concentrated in milk.[9] In a given litter, some kittens can acquire infection in utero, and others will not.[878] Experimental transmission has also been reported after oral,[698,915] intrarectal, and intravaginal inoculation with cell-free or cell-associated virus, and these FIV mucosal transmission models are commonly used to better understand HIV biology and pathogenesis.† The feline female reproductive tract contains CD4+ and CD8+ T cells, B cells, macrophages, and dendritic cells, all known targets of FIV infection. Systemic spread following mucosal routes of inoculation can occur within days.[138,740] FIV infection in cats has also been used as a model of fetal/neonatal HIV infection.[98,152,536]

Despite experimental evidence of FIV transmission via mucosal routes, no evidence exists for this route as being important in maintaining natural infections. Transmission from mother to kittens, in utero or postparturition, is considered a rare event under natural circumstances. Available epidemiologic and antibody surveys, however, do not exclude the possibility of occasional transmission by these routes. High mortality in FIV-positive neonates or rapidly progressive infections, as observed in some experimental studies,‡ may lead to underestimates of in utero and neonatal transmission in natural settings. Additionally, the observation that kittens born to FIV-infected mothers can have FIV provirus in their tissues, but not necessarily their blood, in the absence of detectable antibodies further complicates the understanding of congenital transmission under natural conditions.[8]

*References 411, 531, 574, 766, 938, 1039.
†References 132, 225, 301, 431, 658, 893, 937.
‡References 29, 43, 243, 349, 563, 655, 690, 712, 720, 786, 966, 1032.
§References 71, 594, 613, 645, 712, 716, 966, 1115.
‖References 92, 175, 195, 243, 327, 351, 352, 354, 422, 513, 527, 590, 594, 613, 614, 712, 716, 725, 734, 959, 1097.
¶References 54, 76, 111, 112, 128, 248, 314, 279, 323, 750, 762, 819, 847, 1093.

*References 143, 752, 753, 877, 878, 915, 1068.
†References 30, 33, 124, 440, 529, 740.
‡References 8, 9, 165, 738, 739, 752, 753.

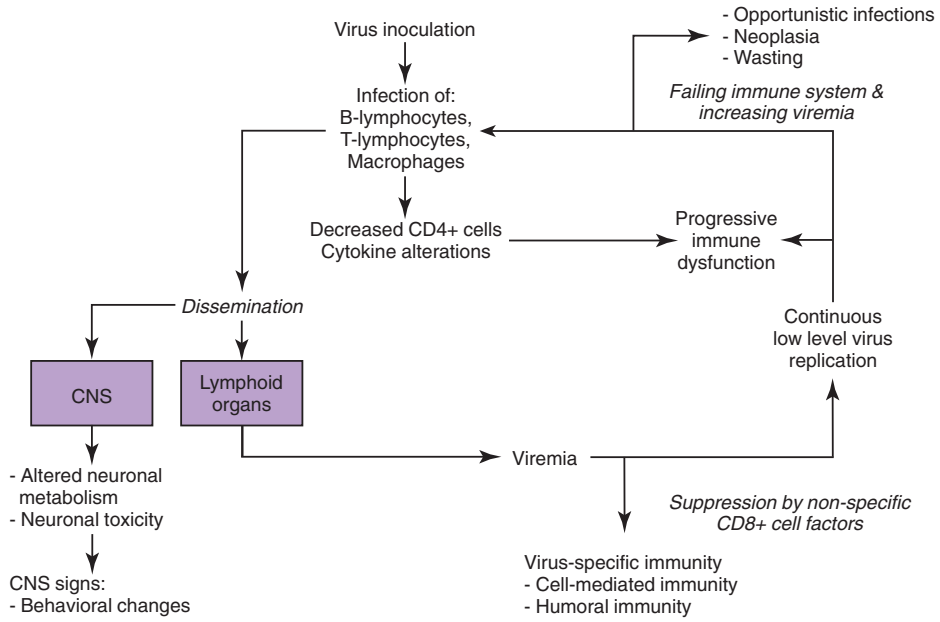

FIG. 12-1 Pathogenesis of FIV infection. *CNS*, Central nervous system; *FIV*, feline immunodeficiency virus.

Infectious virus has been documented in both cell-free and cell-associated fractions of semen from acutely and chronically infected male cats.[486,490] Infection can be established after laparoscopic insemination of queens with semen from infected male cats.[487,488] Although the contribution of seminal transmission to natural infections is unknown, it is likely low.

Horizontal transmission of FIV in multiple-cat households usually is an infrequent event, with some studies suggesting it rarely occurs, but others suggest that horizontal transmission may be common.[3,780] The discrepancy could reflect behavioral differences, such as fighting tendencies, between cats in these households. Cats with positive test results for FIV DNA, but negative test results for FIV-specific antibody (so-called latent infection), have been detected in situations in which the cats with positive DNA test results had been housed in experimental colonies for long periods (months to years) with cats having positive FIV-specific antibody test results (so-called active or viral-productive infection). Interestingly, cats with latent infection were asymptomatic and did not develop typical immunologic abnormalities observed in their cohabitating actively infected cats.[193] Similar cases of clinically healthy cats with latent infections have been observed in other conditions.[8,739] The clinical consequences, if any, of this "latent" type of infection remain unknown at present.

Experimentally, other miscellaneous modes of transmission, such as using suture contaminated with blood from an FIV-positive cat, have been documented.[249] Experimental inoculation of proviral DNA, without complete virus particles, has also produced infection.[796,868,967] Although infection has been established with these modes, natural infections are unlikely to occur through these routes.

PATHOGENESIS

The pathogenesis of FIV infection reflects an interplay of a large number of factors including the age of the animal at the time of infection (young animals develop clinical signs sooner), properties of the FIV isolate (some isolates are inherently more pathogenic than others), the amount of virus used for infection, the route of infection (parenteral versus mucosal or other route), and whether the inoculum is in the form of cell-free or cell-associated (i.e., infected cells) virus.* These factors come together to affect differences in viral kinetics, the character of immune responses to FIV after infection, clinical features, and progression of FIV infection.

After experimental inoculation, viral particles are cleared by tissues rich in macrophages, and viral replication then occurs in target cells of lymphoid organs (thymus, spleen, lymph nodes), and other tissues rich in lymphocytes.† Using polymerase chain reaction (PCR) or viral culture, virus is easily detected in plasma or peripheral blood lymphocytes by two weeks postinfection or even earlier[662] with viremia peaking within several weeks postinoculation.[67,201] FIV also spreads to mononuclear cells (lymphocytes and macrophages) in organs such as the bone marrow, lung, intestinal tract, brain, and kidney.[67,879,898] Results of in vitro studies indicate that FIV-infected dendritic cells can directly transmit virus to CD4+ cells,[319,971] supporting earlier studies that suggested virus-infected follicular dendritic cells in lymph nodes may infect naïve CD4+ cells migrating through the lymph node.[36,1022] A burst of viral replication after activation of CD4+ cells, seen in one study, raises the possibility that FIV takes advantage of dendritic/CD4+ cell interactions to enhance infection.[971]

After peak viremia, circulating virus decreases to low levels as the host mounts an immune response to FIV. A vigorous, but ultimately ineffective, humoral immune response is mounted against the virus (Fig. 12-1). Generally, anti-FIV antibodies first become detectable in experimentally infected cats 2 to 4 weeks postinoculation, although exposure to lesser amounts of virus may delay the appearance of detectable responses.[71] Antibodies are produced against many FIV proteins,[264,286,431,870] especially those of the viral envelope, capsid, and transmembrane proteins.[658] Virus-neutralizing antibodies can be detected with in vitro assays,[40,131,760,1023] but neutralizing antibodies do not efficiently enter cells and therefore do not eliminate virus infection; their role in the suppression of FIV viremia in vivo is not clear.[212,341,460,663,667] Some evidence suggests that the humoral immune response to the virus replicating in infected cats actually is responsible

*References 132, 133, 420, 739, 740, 781.
†References 83, 478, 635, 735, 739, 879, 1024, 1101.

for driving the emergence of FIV variants' resistant to in vitro neutralization.[346] Interestingly, some cats have provirus- (DNA-) positive test results but have FIV antibody–negative test results (see the earlier discussion of latency, under Transmission), suggesting a mechanism by which nonreplicating virus can evade host defense mechanisms.

Although a humoral immune response to FIV is documented, evidence suggests that CD8+ cells play a more important role in suppression of virus production after initial infection.[123,124,179,332,414] Suppression of virus production has been demonstrated in vitro through mechanisms involving both cell-cell interactions and secretion of soluble factors, including interleukin (IL)-16 or others.[579,598,790] The antiviral activity of soluble factors is not restricted to a specific FIV isolate,[162,179,302,416,579] and the mechanism behind its nonspecific, noncytolytic, virus-suppressive activity may be inhibition of viral messenger RNA (mRNA) transcription.[412,418] Inhibitory activity can be detected in some cats approximately 1 week postinfection and before detection of a humoral immune response.[303] CD8+ cell-mediated suppressive activity is maintained through the asymptomatic period, but as infection progresses into the chronic phase, suppressor activity may wane in infected cats.[302,414,419] Virus-specific cytotoxic T-cell activity emerges in the weeks after infection and also plays a role in the host's control of virus.[132] Interestingly, one study described loss of cytotoxic T-cell activity in thymectomized FIV-infected cats, but no alteration in plasma virus loads during the course of the study.[389]

After acute infection and suppression of viremia, cats enter a clinically asymptomatic period of variable duration. This period is not one of true viral latency because FIV production continues in infected cells in tissues, and virus can still be recovered from blood lymphocytes, serum or plasma, cerebrospinal fluid (CSF), semen, and lymphoid tissues.[36,71,357,532] Plasma levels of virus and viral RNA can increase again during the terminal phases of infection, and virus loads may vary from time to time (see Fig. 12-1).[236,357,532]

FIV infects a wide range of cell types in vitro and in vivo (Table 12-1), but cell tropism is isolate dependent.[202,245] Cell tropism is defined by expression of surface proteins that serve as FIV receptors. Unlike HIV, FIV does not use the feline CD4 molecule as a receptor to infect target cells.[436,733] Two proteins with receptor function for FIV have been characterized: CD134 and a chemokine receptor designated CXCR4.* CD134 appears to be expressed more in CD4+ cells than in CD8+ cells, B cells, macrophages, or dendritic cells,[849] and its level of expression in CD4+ cells is increased with activation,[217,849] likely accounting, at least in part, for the well-documented increase in virus production from activated CD4+ cells. Expression of CXCR4 mRNA or antigen can be demonstrated in a large number of cell types known to be susceptible to FIV infection, including lymphocytes, monocytes, macrophages, dendritic cells,[849,1024] and astrocytes. CXCR4 has been detected in intraepithelial mononuclear cells and epithelial cells of the rectum, colon, and female reproductive tract, perhaps accounting for some degree of transmucosal or vertical transmission observed in experimental studies.† CD134 may also play a role in cell infection in nondomestic cat isolates.[952]

The hallmark of FIV pathogenesis is progressive disruption of normal immune function.. Early and persistent immunologic abnormalities that occur after experimental[2,46,1018] and natural[407,737] infection in both domestic and nondomestic felids are decreases in both the number and relative proportions of CD4+ cells (see Fig. 12-1) in the peripheral blood, and in most primary lymphoid tissues examined.[126] Causes of CD4+ cell loss may include decreased production secondary to bone marrow or thymic infection, lysis of infected cells induced by FIV itself (cytopathic effects), destruction of virally infected cells by the immune system, or death by apoptosis.* Apoptosis is a form of cell death that follows receipt of a membrane signal initiating a series of programmed intracellular events that ultimately lead to cell death. Apoptosis of CD4+, CD8+, and B cells has been documented in lymph nodes, spleen, and thymus of FIV-infected cats[421,899,1017]; the degree of apoptosis has correlated inversely with CD4+ numbers and the CD4/CD8 ratio.[421] Increased expression of proteins that mediate apoptosis, as has been demonstrated in lymphocytes from FIV-infected cats, suggests a mechanism by which lymphocytes are susceptible to apoptotic signals in vivo.[127,1036] Proteins coded by the FIV *env* gene are capable of inducing apoptosis in peripheral blood mononuclear cells by a mechanism that requires CXCR4 binding and may be important for inducing apoptosis in bystander cells.[329] Ultimately, loss of CD4+ cells impairs immune responses because CD4+ cells have critical roles in promoting and maintaining both humoral and cell-mediated immunity (CMI). Apoptosis may also contribute to a loss of CD8+ cells on their recognition of FIV antigen.[765]

New insights into FIV-induced immune dysfunction have emerged with examination of a subset of CD4+ cells termed *Treg* (for T-regulatory) cells. Under normal conditions, Treg cells are capable of both antigen-specific and nonspecific suppression of immune responses. In FIV-infected cats, Treg cells with suppressive activity have been documented during early infection[307,680] and in chronically infected cats.[307,789] Treg cells have been shown to inhibit production of interferon (IFN)-γ from CD8+ cells, suggesting that Treg cells could impair immune responses to FIV. Increased activity of Treg cells could also play a role in suppressing immune responses to foreign antigens or pathogens in FIV-infected cats, perhaps accounting in part for some of the susceptibility to infection with other pathogens. In addition, Treg cells are themselves targets for FIV infection,[492,493,680] and an inactive form of Treg cells has been shown to support latent FIV infection in vivo, with activation of viral production provoked by mitogen stimulation.[494] Thus, Treg cells may serve as a FIV reservoir capable of increasing virus production with appropriate activation or stimulation.[494] Experimental inhibition of Treg cells, by use of monoclonal antibody against their $CD25^{hi}$ surface marker allowed FIV-infected cats to produce a more robust humoral response to foreign antigen as compared to cats without Treg cell inhibition.[680a]

Similar to HIV infection in humans, loss of CD4+ cells, in FIV-infected cats, leads to inversion of the CD4/CD8 ratio (see Fig. 12-1), which may occurs weeks to months after infection, depending on the viral isolate studied.[2,1018] An increase in the proportion of CD8+ cells, in particular a population referred to as "CD8+ α-hi, β-low cells," may

TABLE 12-1

In Vivo and in Vitro Cell Tropism of FIV

In Vivo	In Vitro
Lymphocytes (CD4+, CD8+, B cells)[45,62,80,879]	Lymphocytes
Monocytes/macrophages[69,245,450,879,993]	Macrophages[101,653]
Follicular dendritic cells[36,740,879,1022]	Brain endothelial cells[891]
Astrocytes[635]	Astrocytes[244,246]
Brain microglial cells[398]	Brain microglial cells[244,246]
Bone marrow fibroblasts[993]	
Megakaryocytes[69]	
Thymic epithelial cells[879]	
Salivary gland epithelium[772]	

*References 103, 216, 217, 219, 220, 222, 263, 322, 437, 816, 864, 930, 1078–81, 1087, 1088, 1090, 1091.

†References 148, 331, 535, 537, 719, 913.

*References 84, 365, 366, 476, 694, 696, 697, 706, 744, 745, 797, 1017.

be contributing to the inverted ratio.* Although inversion of the CD4/CD8 ratio is a consistent feature of both natural and experimental infections, its use as a prognostic tool for cats is questionable, in contrast to its prognostic value in HIV-infected humans.[88,1061] FIV-infected cats may show severe inversion for prolonged periods without developing clinical signs, and there is no correlation of the ratio with clinical stage of infection or plasma viremia.[358,407]

Several other immunologic abnormalities occur with FIV infection.[1019] Over time, lymphocytes from infected cats lose the ability to proliferate in response to stimulation with lymphocyte mitogens, or recall antigens, and have impaired priming of lymphocytes by immunogens in vitro.† Lymphocyte function may also be impaired by reduced or altered expression of cell surface molecules such as CD3, CD4, major histocompatibility complex II antigens, other co-stimulatory and signaling molecules, and cytokine receptors.‡ Many of these molecules have a critical role in antigen presentation, or amplification and control of immune responses.

FIV infection is associated with disrupted production of cytokines, molecules essential to normal immune function.[1036] Cytokine patterns detected from cultures of FIV-infected lymphocytes are dependent to some extent on the FIV isolate and tissue compartment studied.[205,611] Reported changes in cytokine production in FIV-infected cats, as compared with noninfected cats, have included increased production of IFN-γ, tumor necrosis factor α, IL-4, IL-6, IL-10, and IL-12.[575,599,758,872] Compared to uninfected cats, differences in IL-10/IL-12 ratios have been observed in FIV-infected cats co-infected with *Toxoplasma gondii*,[593] a pattern that could impair development of CMI response to *T. gondii.* Increased IL-10 also has been documented in cats co-infected with FIV and *Listeria monocytogenes*, with FIV-infected cats exhibiting delayed clearance and more severe clinical signs of infection as compared with FIV-negative cats.[201] Altered IL-10/IL-12 ratios have also been noted in bone marrow–derived dendritic cells in response to stimulation of various toll-like receptors,[571] which recognize microbial pathogens and are responsible for initiation of immune responses. Aberrant toll-like receptor responses could also increase the risk for some opportunistic infections.

Alterations in function of nonspecific defenses, such as impaired neutrophil adhesion and emigration in response to bacterial products, have been described in FIV-infected cats,[372,401,540] and these defects in neutrophil function improved with granulocyte-macrophage colony-stimulating factor (GM-CSF) treatment.[401] Natural killer cell activity has been reported as diminished[1119] or increased[1127] in FIV-infected cats, depending on whether the cats were acutely or asymptomatically infected, respectively.

Another manifestation of the immunologic dysregulation observed in many FIV-infected cats is hypergammaglobulinemia, primarily from increases in IgG.[2,299] Hypergammaglobulinemia is usually a polyclonal distribution and the IgG produced is not entirely FIV specific. However, it is a direct consequence of FIV infection because clinically healthy FIV-infected, specific-pathogen free (SPF) cats are also hypergammaglobulinemic.[299] In addition to increased IgG, increased circulating immune complexes have been detected in FIV-infected cats.[660,823] FIV has also been incriminated as causing a delay in the class shift of antibody isotypes from IgM to IgG based on work in cats infected with both FIV and *T. gondii*.[560]

Abnormal neurologic function has been described in FIV-infected cats, and the cat remains a model for the study of acquired immunodeficiency syndrome (AIDS)-related neurologic disease in humans. It is clear from all studies that the origin of neurologic signs in FIV-infected cats is multifactorial.* Although results of some studies have linked neurologic dysfunction and neuronal injury with the amount of virus present in the brain of infected cats,[483,640] results of many others support the hypothesis that neurologic dysfunction and histologic changes in the central nervous system (CNS) are indirect events and not necessarily an immediate consequence of CNS-cell infection or viral replication within the CNS.[672,774,825,886] CNS inflammation is provoked by virus production and increased microglial major histocompatibility complex I expression,[398] and traffic of mononuclear inflammatory cells into the brain may be governed by properties of microglial cells and astrocytes.[443] CD8+ cell-mediated neurologic injury also has been demonstrated.[1128] Experimental infections in vivo have caused brain lesions in the absence of massive CNS infection.[886] Neurotoxins such as glutamate have been implicated as one cause of neuronal loss.[93,102,835,942,1118] Results of other studies have incriminated a role for matrix metalloproteinases, which can break down collagen and alter properties of the blood-brain barrier, leading to altered neuronal cytoskeletal properties or imbalanced neurotransmitters[164,640] in the pathogenesis of neurologic dysfunction in FIV-infected cats.[469,480,484] In vitro and, occasionally, in vivo studies suggest that infected brain astrocytes and macrophages may impair normal CNS-cell metabolism, or may be a source of neurotoxins such as inflammatory cytokines or cell-signaling molecules.† Documented abnormalities of astrocyte and macrophage functions include altered intercellular communication, abnormal glutathione reductase activity that may render cells more susceptible to oxidative injury, alterations in mitochondrial membrane potential that disrupt energy-producing capacities of the cell, and impaired maintenance of intracellular calcium concentrations.[99] Some studies suggest that sequences in the viral envelope protein and levels of chemokine expression within the CNS are important in neurovirulence properties,‡ and others indicate that neurologic-cell dysfunction does not necessarily correlate with envelope-mediated replicative capacity.[82,480] Peripheral nerve dysfunction associated with axonal injury and macrophage activation has also been noted in cats infected with neurovirulent clones of FIV.[516]

The pathogenesis of some clinical features of FIV remains unexplained but, similar to neurologic disease, may result from abnormal function of, or inflammation in, affected organs. Wasting disease has been observed in the absence of obvious causes such as diarrhea or neoplasia. Abnormal renal function and nephritis have also been reported in FIV-infected cats.[586] Many aspects of clinical FIV will reflect the pathogenesis of the secondary diseases such as infections and neoplasms to which FIV-infected cats are considered more susceptible.

CLINICAL FINDINGS

Experimental FIV infection progresses through several stages, similar to HIV-1 infection in humans. Recognized clinical stages in cats include an acute phase, a clinically asymptomatic phase of variable duration, and a terminal phase of infection often referred to as feline AIDS.[274,358] Some investigators have described two other phases in experimentally infected cats in keeping with the terminology for HIV infection: progressive generalized lymphadenomegaly, which follows the asymptomatic phase, followed by AIDS-related complex (ARC).[358,465,466,780] Still other researchers describe a sixth category to encompass miscellaneous diseases such as neoplastic, ocular, and immune-mediated diseases that are observed in some infected cats.[780]

*References 2, 407, 791, 931, 932, 1082.
†References 46, 87, 88, 373, 431, 994, 995, 1020.
‡References 163, 309, 695, 730, 746, 866, 1036, 1083.

*References 173, 295, 296, 444, 671, 1052, 1128, 1131.
†References 82, 101, 192, 534, 673, 731, 1123, 1130.
‡References 102, 364, 400, 480, 482, 639, 731.

Although division of FIV infection into these clinical stages may prove useful from the standpoint of gauging prognosis, no sharp distinction exists between them, and not all stages will be apparent in most naturally infected cats. Furthermore, there is no means to reliably predict the transition from the asymptomatic phase to the ARC or AIDS phase, although one study found that higher levels of viremia during the acute stage of infection were associated with more rapid progression to the terminal phases of the disease.[237] In a study of naturally infected cats, a trend was noted for increasing viremia with progression of clinical signs, with cats in the AIDS category of infection exhibiting higher virus loads than cats in the ARC or asymptomatic phase of infection.[358] In contrast to HIV-infected humans, cats that are classified as being in the AIDS phase (high virus load, severe clinical signs due to secondary infection) may recover and may be asymptomatic again and their virus loads may decrease dramatically. Thus, clinical staging, as in HIV infection, is not valuable in FIV-infected cats.

Clinical signs of FIV infection are nonspecific. Most clinical signs attributed directly to FIV infection, with the possible exception of FIV-induced neurologic disease, likely go unobserved in many naturally infected cats. During acute experimental infection, clinical signs are usually transient and so mild as to go unnoticed. Some cats exhibit fever and malaise. Signs of acute enteritis, stomatitis, dermatitis, conjunctivitis, and respiratory tract disease have been described in experimentally infected cats. Generalized lymph node enlargement is common during acute experimental infection.[739] The acute phase may last several days to a few weeks, after which cats will enter a period in which they appear clinically healthy. The duration of the asymptomatic phase varies and likely depends on factors such as the pathogenic potential of the infecting isolate and the exposure of the infected cats to other pathogens; however, it usually lasts for years. One experimentally infected cat, kept isolated from other cats, had documented viremia for more than 8 years without developing clinical signs.[532] The age of the cat at the time of infection may also influence the length of the asymptomatic stage and the severity of clinical disease, depending on the isolate studied.[781,812] During the later stages of infection, clinical signs are a reflection of opportunistic infections, neoplasia, myelosuppression, and neurologic disease.

Infections with opportunistic pathogens of viral, bacterial (Fig. 12-2), protozoal, and fungal origin have been reported in FIV-infected cats. Few studies, however, have compared the prevalence of most of these infections in corresponding groups of FIV-infected and noninfected cats. In one report, no correlation was found between FIV infection and infection with *Cryptococcus* or *Cryptosporidium*.[711,1063] Another study reported a higher number of fungal genera isolated from the skin, oropharynx, and rectum of FIV-infected cats compared with noninfected cats, but FIV-infected cats had no signs of fungal infections at the time of examination,[647] and no correlation was found between FIV infection and the presence of *Cryptococcus neoformans* or dermatophytes.[941] In one study, antibody prevalence of *T. gondii* was similar in both FIV-infected and noninfected cats, and FIV-infected cats did not have detectable oocyst shedding.[986] In other studies, there has been a high degree of statistical correlation between cats with positive serum FIV-antibody presence and cats with increased *T. gondii*-specific serum antibody titers.[243,627] The prevalence of infection with *Bartonella henselae* or *Bartonella clarridgeiae*, the agents of human cat scratch disease, has not been positively associated in cats with FIV infection [124a,350,402,655,656] as it has been in some studies with feline leukemia virus (FeLV) infection.[124a] In contrast, an increased prevalence of FIV infection has been reported in cats that had serum antibody reactivity to *Bornavirus*,[445] a virus detected in cats in eastern and northern European countries (see Chapter 19), and to orthopoxvirus (see Chapter 17).[1028] A statistical association between FIV infection and

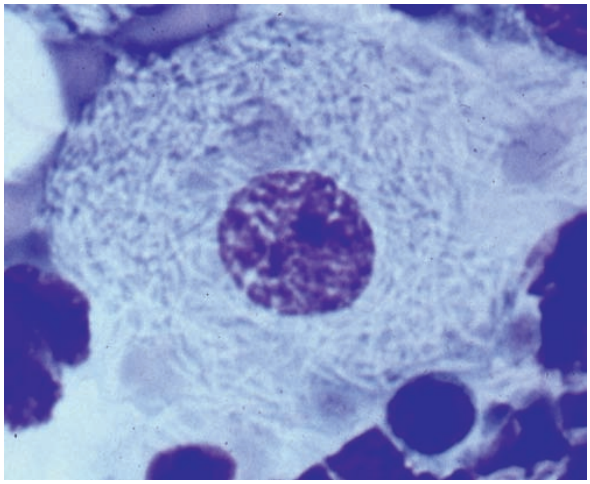

FIG. 12-2 Mycobacteria in a blood macrophage in an FIV-infected cat with disseminated mycobacterial infection. (Courtesy Julie Levy, University of Florida, Gainesville, FL.)

infection with *Mycoplasma haemofelis or Mycoplasma haemominutum*[60,335,637,991] has also been described, but it is not clear if FIV infection is a true risk factor or if infection with both agents reflects common risk factors (e.g., outside lifestyle, male cats). Results of two studies indicated no effect of preexisting FIV infection on numbers of *M. haemofelis*, *M. haemominutum*, or "*Candidatus* Mycoplasma turicensis" organisms in blood, as determined by PCR.[998,1093] Experimentally, FIV infection worsened the respiratory disease observed in an experimental model of acute toxoplasmosis.[196]

Clinical illness may also be caused by opportunistic infections at sites harboring endogenous microflora or by other secondary pathogens that have not been specified. Respiratory disease may be observed in FIV-infected cats and can result from bacterial, fungal, protozoal, or parasitic infections.[57] Diarrhea has been seen in experimentally infected cats in the absence of detectable enteric pathogens. Bacterial overgrowth involving endogenous microflora and inducing inflammatory lesions has been proposed as a possible cause.[770]

Stomatitis is a common finding in FIV-infected cats and can occur during any stage of infection. The pathogenesis of stomatitis is uncertain, although the histopathologic findings of lymphocytes, plasma cells, and variable degrees of neutrophilic and eosinophilic infiltrates suggest either an immune response to chronic antigenic stimulation or immune dysregulation. Stomatitis has not been a consistent finding with FIV infection (see Chapter 88)[846] and is not often seen in experimentally FIV-infected SPF cats, suggesting that exposure to other pathogens may also play a role.[586] Co-infection of FIV-infected cats with feline calicivirus, both experimentally and after natural infection, resulted in more severe oral disease (see Clinical Signs, Chapter 14, and Feline Lymphocytic Plasmacytic Ulceroproliferative-Gingivostomatitis (Faucitis), Chapter 88).[857,1005] Odontoclastic resorptive lesions have been reported with higher prevalence in cats experimentally infected with FIV, as compared with noninfected cats; such lesions have been speculated to be a consequence of gingivitis or stomatitis present in the cats.[408]

Neurologic signs have been described in both natural and experimental, and acute and terminal FIV infections.* Neurologic impairment after FIV infection appears to be isolate dependent.[834] The most common neurologic signs observed are behavioral changes. Other abnormalities that have been described include seizures, paresis,

*References 1, 80, 246, 277, 794, 815.

multifocal motor abnormalities, impaired learning, and disrupted sleep patterns.[367,839,975] Neurologic signs may improve if they occur during the acute stage of infection, although residual deficits are possible. Abnormal forebrain electrical activity and abnormal visual and auditory-evoked potentials have also been documented in cats that appeared otherwise normal.[52,796,812,814] Less commonly, secondary infections such as feline infectious peritonitis, toxoplasmosis, or cryptococcosis cause the observed neurologic deficits.

FIV-infected cats can develop ocular disease,[275,559,755,1094] and abnormalities may be found in both anterior and posterior segments. Anterior uveitis may result from secondary infections such as toxoplasmosis or may be directly related to FIV infection.[275,755,1094] Glaucoma, with and without uveitis, has also been described.[275,755,1094] Posterior segment changes that may be seen include pars planitis (an infiltration of leukocytes, mainly plasma cells, into the vitreous behind the lens), focal retinal degeneration, and retinal hemorrhages.[275,1094]

Neoplasia is a common reason that FIV-infected cats are brought to a veterinary clinic. Statistically, FIV-infected cats are much more likely to develop lymphoma or leukemia compared with noninfected cats.[144,818] Lymphomas, leukemias, and a variety of other tumor types have been reported in association with FIV infection.* Most lymphomas in FIV-infected cats are B-cell tumors.[144,327,818,1006] FIV provirus is only occasionally detected in tumor cells,[62–64,1066] suggesting an indirect role for FIV in lymphoma formation, such as decreased cell-mediated immune surveillance or chronic B-cell hyperplasia.[64,270] However, clonally integrated FIV DNA was found in lymphoma cells from one cat that had been experimentally infected 6 years earlier,[62,63] raising the possibility of an occasional direct oncogenic role of FIV. The prevalence of FIV infection in one cohort of cats with lymphoma was 50%,[327] much higher than the FIV prevalence in the population of cats without lymphomas, which is supportive of a cause and effect relationship between FIV and feline lymphoma.

In the terminal phase of infection, a wasting syndrome may occur. If experimentally infected with some particularly pathogenic FIV isolates, SPF cats have developed a terminal wasting syndrome within 6 to 8 weeks postinfoculation.[235,237]

DIAGNOSIS

Clinical Laboratory Findings

A large number of clinicopathologic abnormalities have been described in FIV-infected cats, but none are specific or pathognomonic for infection. During the acute phase of infection, cats may exhibit neutropenia and lymphopenia, which resolve as the cat progresses to the asymptomatic phase of infection.[609,648] During the asymptomatic phase of infection, results of complete blood cell count (CBC) and biochemical analyses are often within reference limits,[523,609,612,648,925] but leukopenia may be encountered.[29,925] Clinically ill FIV-infected cats may have a variety of cytopenias. Lymphopenia, caused mainly by decreases in CD4+ cells, is most common (see Pathogenesis). Flow cytometric analysis of peripheral blood lymphocytes, available at many veterinary teaching hospitals, may demonstrate inverted CD4/CD8 ratios. Inverted CD4/CD8 ratios are only consistent with, but not pathognomic for, FIV infection.

Anemia and neutropenia may also be observed, although these abnormalities may be as much a reflection of concurrent disease as direct effects of FIV infection itself.† Anemia, which usually is nonregenerative, leukopenia and neutropenia, thrombocytopenia, or combinations of cytopenias including pancytopenia have been observed in asymptomatic FIV-infected cats in the absence of other identifiable causes.[29,325,351,378,781] A study of a high number (3784) of client-owned field cats compared hematologic parameters in FIV-infected or FeLV-infected and uninfected cats.[351] Anemia and thrombocytopenia were not significantly more common in FIV-infected than uninfected cats. Only neutropenia was significantly more often present, in about 25% of FIV-infected cats. Abnormalities in morphology of erythrocytes and platelets, and platelet-bound antibodies in thrombocytopenic FIV-infected cats, have been described.[325,533] Soluble factors have been shown to inhibit bone marrow function in FIV-infected cats, and bone marrow infection has been associated with decreased ability to support hematopoietic potential in vitro or has been proposed as a mechanism underlying the development of cytopenias.[325,993]

Abnormalities of the biochemical profiles of FIV-infected cats typically are few. Some cats have an increase in total protein caused by hyperglobulinemia, which is usually polyclonal.[351,684,850] Azotemia has been reported in FIV-infected cats in the absence of other detectable causes of renal disease,[586,822,1008] and a study demonstrated that cats younger than 11 years of age with chronic kidney disease were more likely to have positive FIV serum antibody results,[1075] but linking FIV as a definitive cause of renal disease awaits clarification. Other biochemical abnormalities, when found, will usually reflect concurrent disease. In one study, after 9 months of experimental FIV infection, cats had in addition to hyperglobulinemia, increased glucose, triglyceride, urea, and creatinine concentrations and reduced serum cholesterol levels.[409] In a study in naturally infected cats, only hyperglobulinemia was a consistent feature.[351] Prolongations of the activated partial thromboplastin time have been reported in FIV-infected cats in the absence of other obvious causes of coagulopathy, although the prolongations were mild and not clinically apparent.[378]

Few descriptions of CSF changes have been provided in FIV-infected cats with neurologic disease. Cellular pleocytosis and increases in concentrations of IgG in the CSF have been reported,[246] and increases in cell number and total protein in CSF is not unreasonable. Viral RNA can be found in the CSF of some cats and suggests parenchymal infection.[886]

Antibody Testing

A definitive diagnosis of FIV infection is made most commonly by detection of FIV-specific antibodies in blood. Most cats produce antibodies within 60 days of exposure, but development of detectable antibodies may be considerably delayed in some cats. Because FIV produces a persistent infection from which cats do not recover, infected cats usually develop high amounts of FIV-specific antibodies. Detection of antibody has historically been synonymous with infection. Antibodies are usually detected in practice by either ELISA or rapid immunomigration-type assays, which are widely available and easy to use (see Web Appendix 6).[387,824] These in-clinic test kits detect antibodies to different viral antigens, most commonly p24. One study has suggested improved sensitivity when assays include a combination of FIV proteins.[881]

Available point-of-care FIV tests are highly sensitive and specific. Several studies showed that the performance of point-of-care FIV/FeLV test kits for the detection of FIV infection is excellent.[387,798,895] Because the consequences of a positive test result are potentially clinically important, confirmatory testing is still recommended, especially in low-risk and asymptomatic cats for which the risk of a false-positive result is higher.[468] False-positive results still may occur, especially in cats from areas with low prevalence of infection,[387,388,874,895] and technical error and use of whole blood rather than serum in some of the test kits have been incriminated as causes of false-positive results.[387,388] Negative test results are highly reliable

*References 50, 128, 145, 179, 294, 456, 818.
†References 114, 850, 923, 924, 969, 972.

because of the high sensitivity of the tests and the low prevalence of infection in most populations.

There are several options for confirmation of a positive screening test. Virus culture is the gold standard for identification of FIV infection, but is not routinely available. Alternatively, a second ELISA-based antibody test can be performed, preferably using a point-of-care test from a different manufacturer.[387] Western blot and indirect fluorescent antibody methods detect antibodies against a range of FIV antigens but were found to have lower specificity than ELISA screening tests in one study, especially in those cats vaccinated against FIV infection.[589]

Although very convenient, and highly reliable in most situations, antibody testing to establish a definitive diagnosis of FIV infection also has some pitfalls. Antibody tests have to be interpreted carefully in kittens less than 6 months of age. Kittens up to 6 months of age can have anti-FIV antibodies passively acquired from their dams that are infected or have been vaccinated.[636] Rarely, kittens become infected from their mothers under natural circumstances; therefore, most kittens that initially have positive test results will eventually have negative results when maternal antibodies wane. Retesting these kittens after 6 months of age is advised. If the second test result is negative, the earlier positive result was likely the result of maternal antibody. If the result remains positive, the kitten is likely infected. If a kitten less than 6 months of age has a negative antibody result, it is likely to be truly negative. However, there is a small chance that the kitten has not had time to develop a detectable antibody response. Therefore, kittens with unknown background should have antibody testing repeated after 60 days. If the results are still negative, the kitten is unlikely to be infected. Current American Association of Feline Practitioners guidelines (Box 12-1) recommend that every kitten, independent of age, should be tested. Untested kittens could be a source of infection for other cats if they are not identified and segregated. Also, compliance by both owners and veterinarians with retroviral testing recommendations remains low, and delaying testing of newly acquired kittens would likely result in a large number of cats never receiving FIV tests.[354]

Cats in an early phase of infection may have negative antibody test results. Thus, when the results of antibody testing are negative but recent infection cannot be ruled out, testing should be repeated a minimum of 60 days after the last potential exposure. Although most cats develop a detectable antibody response within 60 days of initial infection, in some experimental studies, antibody development has not been observed until 70 days postinoculation, and occasionally in animals when the time delay has been 6 months or longer.[412,780] These observations from experimental infections suggest that a single negative test result may, in some instances, be insufficient to discriminate recently infected animals from uninfected animals. Furthermore, antibody increases may not occur at all in rapidly progressive infections.[754] FIV-specific antibodies are readily detected in blood of most cats throughout the asymptomatic phase of infection, but asymptomatic kittens with very low antibody titers or with no detectable antibodies have been observed after experimental infection. In these kittens, evidence of FIV infection could only be demonstrated by virus culture or PCR.[739,754] Furthermore, because of debilitation of the immune system, some cats entering the terminal phase of infection may have a reduction in detectable antibody,[236] although this is very unlikely in natural infections. For such cats, Western blots may show FIV-specific antibodies not detected by some immunochromatographic tests.

The availability of the FIV vaccine in the United States has created an additional complication for the ability of veterinary practitioners to diagnose FIV infections based on antibody detection assays.[178] Vaccinated cats produce antibodies that cannot be distinguished from antibodies induced by natural infection by any commercially available antibody test, including Western blots.[590] These antibodies are usually detected within a few weeks of vaccination and have been shown to persist for more than 3 years in some cats.[591] Antibodies are also passed to kittens that nurse on vaccinated queens. Passively acquired vaccine-associated antibodies persist past the age of weaning (8 weeks) in more than half of kittens.

An experimental method of ELISA testing that detects antibodies to multiple FIV antigens has been developed in Japan.[544] Using this method to test serum samples from cats in the United States and Canada, researchers were able to distinguish FIV-vaccinated cats from FIV-infected cats with a high degree of accuracy.[591] This test, however, is not yet commercially available.

In adult cats that are known with certainty to not have been vaccinated, antibody tests are still reliable to diagnose FIV infection. Misdiagnosis of FIV in uninfected cats (e.g., positive results in vaccinated cats) may lead to the euthanasia of vaccinated cats or of kittens born to vaccinated queens. This issue is especially problematic in shelter medicine, given that confirmatory testing is frequently expensive and impractical. Experimental evidence also suggests that superinfection with different subtypes is possible (i.e., inconsistent cross-protection), and thus, a cat vaccinated with a dual subtype vaccine could still be infected with a subtype not included in the vaccine. Antibody assays are not helpful in determining the infection status of such cats. Thus, in cats with unknown vaccination status, it may be difficult to determine if a positive FIV antibody test result means the cat is truly infected with FIV, is vaccinated against FIV but not infected, or is vaccinated against FIV and also infected.

In exotic cats, FIV-related virus infections that occur with high prevalence are commonly diagnosed using commercial FIV antibody detection assays. Data from one study indicated that commercial domestic cat FIV-based immunoblots and ELISAs provided a fair ability to recognize FIV antibody-positive serum specimens from

BOX 12-1

Guidelines for FIV Testing in Cats

Testing cats for FIV is recommended for the following circumstances:

- All cats that are sick, even if they have tested negative in the past
- All cats that are to be adopted; cats that are negative should be retested again after 60 days
- All cats that are of unknown status
- All cats that have risk factors for recent exposure, or are suspected or known to have been recently exposed (retesting of negative cats after 2 to 3 months may be needed to allow time for seroconversion)
  - Has bite or fight wounds
  - Has a history of unsupervised outdoor activity
  - Resides with, or has been exposed to, a cat whose FIV status is unknown
  - Resides with a cat with positive FIV antibody test result (annual testing recommended unless isolated from the cat with positive FIV antibody test results)
- All cats that are to be vaccinated for FIV (negative test results advised before vaccination)
- Cats that are to be used for blood or tissue (e.g., kidney) donation

FIV, Feline immunodeficiency virus.
Note: Intermittent retesting is not considered necessary for cats with confirmed negative infection status unless there has been an opportunity for exposure, or illness develops.
Modified from Ref. 260.

exotic felids when compared with species-specific immunoblots for screening bobcats and ocelots, but were not that reliable in pumas.[316]

Virus Detection

With the introduction of the FIV vaccine and the associated problems of interpreting antibody test results in vaccinated animals, other methods have been employed to help confirm FIV infection. Cats infected with FIV have low viral loads throughout most of their lives so that it has not been possible to devise ELISA-screening assays based on antigen detection as for FeLV infection. Detecting infection by more sensitive means, such as virus isolation and PCR, has been suggested to determine a cat's true infection status. Although classical virus detection by blood cell culture and virus isolation from plasma is possible over the whole infection period, it is time consuming, expensive, and requires expertise. Therefore, this method is not practical for routine diagnosis.

PCR methods have been sensitive and specific when used in experimentally infected cats. The marketed vaccine should not result in provirus production and thus should not interfere with PCR assays that detect viral DNA.[1033] However, potential conflicts may arise in the future with PCR tests, depending on the level of attenuation of a vaccine. PCR requires sophisticated equipment and so is only performed in specialized laboratories. PCR tests are not standardized across laboratories. False-positive results are possible with FIV PCR as they are with all PCR assays.[28,79] The marked variability of the FIV genome has raised concerns about the ability of individual PCR methods to detect all FIV variants, or isolates of other subtypes.[16] PCR reagents, including primer and probe sequences, are often selected based on genetic sequences of a few well-characterized FIV strains and so may not detect all isolates. Additionally, some laboratory cats with documented FIV infection have insufficient circulating provirus copies for detection by conventional PCR.[8,586]

Although PCR has been widely promoted as a method to determine a cat's true status, investigation of the sensitivity and specificity of the FIV-PCR tests offered by some laboratories has shown widely variable results. In one study, test sensitivities ranged from 41% to 93%, and test specificities ranged from 81% to 100%.[180] Unexpectedly, false-positive results were higher in FIV-vaccinated cats than in unvaccinated cats, raising further concerns about the reliability of PCR testing. Failure to identify infected cats (e.g., PCR-negative results caused by strain variations) may lead to inadvertent exposure and transmission of FIV to uninfected cats. Thus, PCR cannot be recommended as a test for definitive diagnosis of FIV infection in the field. However, research is focusing on improving the diagnostic accuracy of PCR for FIV. Quantitative PCR detection of host-cell integrated FIV DNA of the various subtypes was successful in distinguishing between vaccinated and infected cats; however, the positive detection rate in infected cats was only 60%.[1063a]

Diagnostic Recommendations

In summary, the FIV antibody status of all cats evaluated in a practice should be established because there are health consequences of infection that influence patient management, both in illness and for wellness care.[584] Failure to identify infected cats may lead to inadvertent exposure and transmission to uninfected cats. Misdiagnosis of infection in uninfected cats may lead to inappropriate changes in lifestyle or even euthanasia. Even cats living in single-cat households should be tested for several reasons: their FIV status may influence their health, other cats may join the household in the future, and FIV-positive cats confined indoors may escape and expose other cats. Additionally, cats may require retrovirus testing at different times in their lives. The American Association of Feline Practitioners guidelines for testing cats for FIV are noted in Box 12-1.

PATHOLOGIC FINDINGS

Numerous pathologic changes occur as a result of FIV infection. The lymph nodes of experimentally FIV-infected cats may be hyperplastic during the acute phase of infection, and those in the terminal phase of infection may have disruption of normal architecture with loss of follicles and cellular depletion.[272,739,1110] Dysplastic changes have been reported in the bone marrow of FIV-infected cats, along with the appearance of granulocytic hyperplasia and the formation of marrow lymphoid aggregates.[105] Inflammation in the respiratory and gastrointestinal (GI) tracts has also been seen. Infected cats can develop lymphoid interstitial pneumonitis, characteristic of lentiviral infections in other species.[140] Renal lesions found in FIV-infected cats include glomerulosclerosis and tubulointerstitial infiltrates.[586,822] The existence of an FIV-associated myopathy characterized by infiltrates of lymphocytes and plasma cells in perivascular and pericapillary areas, and myofiber infiltrates of skeletal muscles in conjunction with myofiber necrosis have also been documented. Affected animals, however, did not exhibit clinical signs of myopathy.[811] Experimentally infected cats that develop neurologic disease may have lymphocytic infiltration of perivascular areas (Fig. 12-3).[1] Loss and reorganization of neurons, axonal sprouting, and gliosis are described in FIV-infected cats; many of these changes can be found in cats without obvious evidence of clinical signs of infection.[674,685,686,820] Giant cell formation has also been reported.[367] Some of the common pathologic abnormalities observed in FIV-infected cats are listed in Table 12-2.

THERAPY

In most naturally infected cats, FIV does not directly cause a severe clinical disease. With proper care, FIV-infected cats can live many years with a high quality of life and, in fact, may die in older age from causes unrelated to FIV infection. Long-term monitoring of a 26-cat household with endemic FeLV and FIV infections revealed that all FeLV-infected cats died within 5 years, but FIV infection did not affect survival in this group.[3] A large study compared the survival of more than 1000 FIV-infected cats to more than 8000 age- and sex-matched uninfected control cats.[592] In FIV-infected cats that were not euthanized at the time of diagnosis, median survival was 4.9 years, compared to 6.0 years for control cats. In contrast, in FeLV-infected cats median survival was only 2.4 years. Thus, with proper care, most FIV-infected cats may live for several years with good quality of life,[352] and a decision for treatment or for euthanasia should not necessarily be based solely on the presence of FIV infection.[423]

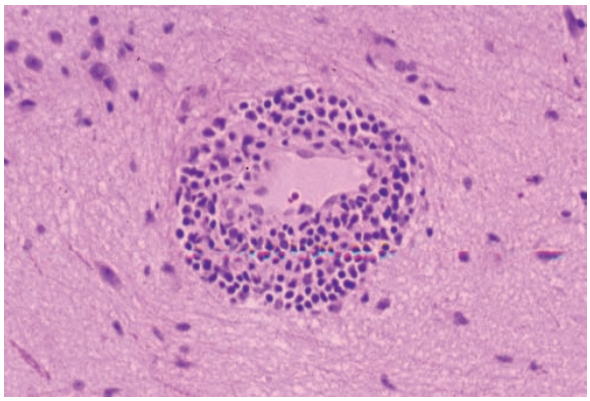

FIG. 12-3 Perivascular inflammation in the CNS from a cat with FIV infection and polioencephalomyelitis (H&E stain, ×100). (Courtesy Bob English, North Carolina State University, Raleigh, NC.)

TABLE 12-2

Pathologic Abnormalities Described in FIV-Positive Cats[a]

Area	Abnormality
Lymph node	Follicular involution Follicular hyperplasia Follicular plasmacytosis
Thymus	Cortical involution, atrophy Lymphoid follicular hyperplasia and germinal center formation
Intestinal tract	Villous blunting Pyogranulomatous colitis Lymphoplasmacytic stomatitis
Liver	Periportal hepatitis
Bone marrow	Myeloid hyperplasia, lymphoid aggregates
Kidney	Interstitial nephritis Glomerulosclerosis
Central nervous system	Perivascular cuffing Gliosis Myelitis Decreased neuron density, axonal sprouting, vacuolar myelinopathy
Lung	Interstitial pneumonitis, alveolitis
Skeletal muscle	Lymphocytic myositis Myofiber necrosis Perivascular cuffing

FIV, Feline immunodeficiency virus.
[a] From references 67, 69, 105, 115, 143, 146, 206, 235, 246, 282, 455, 478, 635, 661, 674, 685–687, 811, 820, 822, 823, 865, 1110.

It is important to recognize that FIV-infected cats are subject to the same diseases that befall cats free of retrovirus infections, and that a disease diagnosed in a FIV-infected cat may in many cases not be related to the retrovirus infection. Nonetheless, specific treatment of FIV has been an area of investigation not only for the potential of helping FIV-infected cats, but also for the potential benefits to HIV-infected humans. Treatment includes antiviral chemotherapy, immune modulatory therapy, and husbandry and supportive care.

Antiviral Chemotherapy

Highly active antiretroviral therapy cocktails are the mainstay of treatment in HIV-infected patients today and result in longer survival times and improved quality of life. Most antivirals developed for lentivirus infections are specifically intended for treatment of HIV infection, and few can be used to treat FIV infection despite similarities in the sensitivity of some enzymes of FIV and HIV to inhibitors. In cell culture, many compounds are active against FIV, and many FIV treatment studies screen new compounds in vitro or in vivo to document a potential benefit for HIV-infected humans. Potentially confounding interpretation of in vitro studies is the observation that results of treatment of FIV-infected cell cultures with nucleoside analogue reverse transcriptase (RT) inhibitors varied with the cell culture system used.[1045] Although antiviral therapy has been used in FIV-infected cats, the drugs actually available to treat cats are limited, tend to be more toxic in cats than in humans, and have few controlled studies to support their clinical use. Refer to the Drug Formulary in the Appendix, and Chapter 2 for more information on drugs discussed in this section.

Treatment with nucleoside analogues such as zidovudine (AZT), alone or in combination with other drugs, has been investigated in vitro and in vivo.* AZT blocks lentivirus-RT activity and is the most thoroughly studied anti-FIV drug. AZT is integrated into the developing DNA strand, thus inhibiting infection of new cells. AZT reduces plasma virus load, improves the immunologic and clinical status of FIV-infected cats, increases quality of life, and prolongs life expectancy.[380,381] Depending on the study, treatment with AZT before inoculation with FIV does not prevent infection or virus replication in target tissues but delays the onset of detectable viremia and some of the immunologic changes.† AZT improves neurologic abnormalities in FIV-infected cats.[586] Regression of stomatitis has been observed in placebo-controlled studies of naturally infected cats treated with AZT.[261,384,386] It also improved CD4/CD8 ratios. As is the case with HIV, evidence from in vitro studies suggests that FIV can become resistant to AZT and other nucleoside analogues.[353,853,854] In fact, a single-point mutation in the FIV gene can create resistance to one or more of the nucleoside analogues.[668,954] In vivo studies of FIV-infected cats treated with nucleoside analogues have associated mutations in the FIV RT gene with such therapy.[654] During treatment with AZT, regularly performed CBCs are necessary because nonregenerative anemia is a common side effect, especially if higher dosages are used.[261,956] CBCs should be performed weekly for the first month. Some cats may develop a mild decrease of hematocrit (HCT) initially in the first 3 weeks that resolves even if treatment is continued. If the HCT drops below 20%, discontinuation of treatment is recommended, and anemia usually resolves within a few days. If values are stable after the first month, a monthly CBC is sufficient. Cats with bone marrow suppression should not be treated because of the risk of life-threatening anemia. In cats with concurrent chronic renal failure, the dose should be reduced to avoid toxicity from accumulation of the compound. Studies in cats treated with AZT for 2 years showed that AZT is well tolerated in most FIV-infected cats. A dosage regimen for AZT is listed in Table 12-3. In contrast to the nucleoside RT inhibitors, nonnucleoside RT inhibitors have no activity against FIV.[31]

Given the role of the CXCR4 molecule in viral transmission, CXCR4 ligands such as stromal derived factor-1 have been developed and investigated for their anti-FIV potential, but inhibitory activity has been inconsistent, likely reflecting the role of other molecules in cell infection.[271,426] In vitro studies have demonstrated antiviral activity of peptide antagonists of CXCR4.[692] Bicyclams selectively block CXCR4 receptors on feline and human cells. When bound to CXCR4, the bicyclams prevent the interaction of this receptor with ligands, such as HIV or FIV, thereby inhibiting the entry of these viruses into the cell.[242,263,911] Plerixafor (AMD3100) is the prototype compound among the bicyclams. It effectively inhibited FIV replication in vitro[263] and significantly reduced the virus load of naturally FIV-infected cats without inducing resistance in a placebo-controlled double blind clinical trial.[981] The use of AMD3100 might be a viable approach in the treatment of FIV-infected cats. Magnesium and calcium levels should be monitored regularly during treatment (see Plerixafor, Chapter 2).

Inhibition of infection via blockade of CD134 is also generating some study.[1089] The potential promise in targeting CD134 is illustrated in a study demonstrating a correlation with lower virus load and better health status in FIV-positive cats with high concentrations of antibody to CD134.[359]

Proteinase inhibitors, which have been used to successfully control illness in HIV-infected humans, are strongly retrovirus species-specific. An experimental drug developed for FIV, known as TL-3, was effective in prevention and resolution of FIV-induced CNS

*References 90, 261, 379, 380, 384, 385, 668, 795, 956, 1037.
†References 261, 384, 385, 390, 391, 675, 795.

TABLE 12-3

Drug Therapy, Used with Variable Efficacy, to Treat FIV Infection

Drug	Dose[a] (mg/kg)	Route	Interval (hours)	Duration (weeks)
ANTIVIRAL				
Zidovudine[b]	5 mg/kg	PO, SC[c]	12	prn
CYTOPENIAS				
Erythropoietin	100 IU/kg	SC	48	2[d]
Granulocyte colony-stimulating factor	5 µg/kg	SC	12	1–2
Human interferon-α	50 IU total per cat	Topical oral mucosa	24	7[e]
Feline interferon-ω	106 IU/kg	SC	24	5[f]
STOMATITIS				
Metronidazole	5 mg/kg	PO	8	2–4
Clindamycin	5–11 mg/kg	PO	12	2–4
Prednisone, Prednisolone	1–2 mg/kg	PO	24	2–4
Bovine lactoferrin	40 mg/kg	Topically to oral cavity	24	prn

FIV, Feline immunodeficiency virus; *PO*, by mouth; *prn*, as needed; *SC*, subcutaneously. See text and Chapter 2 for more information on efficacy.
[a]Dose per administration at specified intervals. See the Drug Formulary in the Appendix, for additional information.
[b]Monitor CBC regularly for Heinz body anemia.
[c]For PO, administer in gelatin capsules with specific calculated dose; for SC, dilute lyophilate in 5 mL sodium chloride.
[d]Until desired hematocrit is reached.
[d]Has been given in a continuous fashion or for 7 days on and 7 days off for 6 months followed by a 2-month break, then repeat treatment.
[d]This 5-day course of treatment was repeated beginning on days 0, 14, and 60.[214]

disease, but treatment had to be continued to maintain remission.[395,454] Newer HIV drugs such as integrase strand transfer inhibitors have impaired FIV replication abilities in vitro,[906] but clinical utility has yet to be demonstrated.

Additional compounds (see Chapter 2) with a wide variety of structure and function have been evaluated; however, their clinical efficacy has not been established.*

Immunomodulatory Therapy

Treatment strategies centering around bolstering immunity in FIV-infected cats have been attempted. Some have used FIV antigen-stimulated lymphocytes or FIV-infected dendritic cells without obvious benefit in long-term viral parameters.[306,321] Immunomodulators or immunostimulatory agents such as acemannan, *Staphylococcus* protein A, and *Propionibacterium acnes* have been advocated for use in retrovirus-infected cats to restore compromised immune function, thereby allowing the patient to control viral burden and recover from associated clinical syndromes. Most reports that appear in the veterinary literature are difficult to interpret because of unclear diagnostic criteria, absence of clinical staging or follow-up, the natural variability in the progression of infection, the lack of placebo-control groups, the small number of cats used, and concurrent administration of other supportive treatments.[586] There is no conclusive evidence from controlled studies to show that immunomodulator or alternative drugs have any beneficial effects on the health or survival of asymptomatic or symptomatic FIV-infected cats.

Treatment with antioxidative substances has been suggested for FIV-infected cats as glutathione peroxidase blood activities decrease significantly during chronic FIV infection, potentially suggesting oxidative stress in infected cats.[1072] Oral supplementation of the antioxidant superoxide dismutase in six acutely experimentally FIV-infected cats increased the CD4/CD8 ratio, indicating a positive effect on this immune parameter, but did not influence virus load.[1073]

IFNs are immunomodulators, but type 1 IFNs (e.g., human IFN-α, feline IFN-ω) also act as true antivirals thus having both immunomodulatory and direct antiviral effects (see Chapter 2). Human IFN-α has been used in cats with FIV infection.[784,871] Parenteral administration of IFN-α is more likely to produce an antiviral effect than is oral administration.[908] Human IFN-α can be given in high doses (10^5 to 10^6 IU/kg) parenterally for up to 6 or 7 weeks, after which cats are likely to develop antibodies.[1120] Alternatively, human IFN-α given orally in low doses, as is used by many veterinarians to treat retrovirus infections, is likely not absorbed but rather destroyed in the GI tract so measurable serum levels do not develop. Oral IFN-α may, however, act through stimulation of local lymphoid tissue in the oral cavity. Low-dose oral (50 IU every 24 hours) human IFN-α has been given on the oral mucosa for 7 days on alternating weeks for 6 months, followed by a 2-month break, and then repetition of the 6-month treatment. This regimen was used in a trial with clinically ill cats naturally infected with FIV. All but 1 of the 24 cats in the treatment group were alive at 18 months compared to only 1 of the 6 placebo-treated cats. Although there was a significant difference in the survival rate and in the clinical condition of the cats, there was no difference in virus load, suggesting rather that the improvement was due to treatment of opportunistic infections.[784] This study would support the use of low-dose oral human IFN-α in FIV-infected cats. However, it is possible that nonspecific stimulation of the immune system in FIV-infected cats may be counterproductive as it can lead to progression of FIV infection.

Feline IFN-ω has been available as a recombinant product for use in some countries (e.g., in Europe and Japan) for several years. Feline IFN-ω effectively inhibits FIV replication in vitro[992] but has not shown benefit in FIV-infected asymptomatic cats.[149] One field study has been performed in 62 naturally FIV-infected cats treated with IFN-ω at 10^6 IU/kg subcutaneously every 24 hours on 5 consecutive

*References 90, 229, 232, 259, 260, 342, 345, 385, 454, 511, 512, 568, 633, 641, 642, 657, 670, 691, 702, 706, 707, 727, 732, 747, 782, 920, 1029, 1035.

days in a placebo-controlled multicenter trial. This study did not show significant changes in survival rate in treated cats when compared to a placebo group, although some clinical improvement was noted.[214] The treatment regimen used, however, was probably too short to treat chronic FIV infection. Feline IFN-ω can be used parenterally for an unlimited period of time because cats do not develop anti-IFN antibodies. Other trials will be needed to assess the efficacy of feline IFN-ω against FIV infection. Other IFNs have been investigated only in vitro. IFN-ω has shown some efficacy against FIV in vitro, but in vivo studies have not been reported.[828]

Management of FIV-Infected Cats

In all cats, FIV status should be known because FIV infection affects long-term management. Management of FIV-infected cats should differ from that of noninfected cats. The strategy most likely to prolong the life of an FIV-infected cat is keeping the cat strictly indoors.[3] Such a strategy prevents both exposure of the immunosuppressed cat to infectious agents carried by other animals and spread of FIV to other cats. Secondary diseases cause the majority of health problems in FIV-infected cats. These secondary diseases also cause clinical signs in FIV-infected cats, influence the clinical course of FIV infection, and play a role in the progression of FIV infection. Good nutrition, husbandry, and an enriched lifestyle are essential to maintaining good health. Cats should be fed a nutritionally balanced and complete feline diet. Raw meat and dairy products should be avoided because of the risk of foodborne bacterial and parasitic infections. A program for routine control of GI parasites, ectoparasites, and heartworms, where applicable, should be implemented.[584] Regular (at least semiannual) veterinary examinations should be encouraged to promptly detect changes in health status. Annual evaluation of a CBC, biochemistry profile, and urinalysis have also been recommended.[584,858] If FIV-infected cats are sick, prompt and accurate identification of the secondary illness is essential to allow early therapeutic intervention and a successful treatment outcome. Therefore, intensive diagnostic testing should occur earlier in the course of illness for a cat with positive FIV antibody test results than might be recommended for an uninfected cat. In addition to a thorough physical examination and laboratory database, thoracic and abdominal radiographs or abdominal ultrasonography may be required to identify the presence of secondary disease. Consideration should be given to cytology and culture of pertinent samples (e.g., urine, blood, effusions, tracheal wash) as additional diagnostic tests and as guides to pharmacologic choices. Cats with cytopenias may require bone marrow aspiration or biopsy to identify underlying causes.

When underlying infections are identified in FIV-infected cats, treatment with appropriate antibiotics or antifungals is encouraged because no evidence suggests that the FIV-infected cat is incapable of responding to treatment. More prolonged courses of treatment may be required in some animals. Systemic fungal infections in FIV-infected cats should be treated the same as in noninfected cats, but FIV-infected cats with dermatophyte infections should not be treated with griseofulvin because this drug has been associated with the development of severe neutropenia in cats with naturally acquired FIV infection.[922] Itraconazole is useful as a systemic agent for treatment of dermatophytosis (see Chapter 56 and the Drug Formulary in the Appendix).

Some FIV-infected cats may have anemia secondary to bone marrow suppression. In cats in which underlying causes of anemia are not found, consideration may be given to treating with erythropoietin (100 IU/kg, subcutaneously, every other day until desired HCT is reached, then as needed to maintain the HCT). In one study of the use of hematopoietic factors, administration of recombinant human (rHu) GM-CSF, but not erythropoietin, was associated with increases in virus production both in vitro and in vivo.[26] Another study, investigating the benefits of rHu granulocyte colony-stimulating factor (G-CSF) in FIV-infected cats, found no changes in infection status, but development of neutralizing, cross-reactive antibodies to feline G-CSF[792] associated with lowering of neutrophil numbers. Therefore, potential benefits of administration of rHuGM-CSF to neutropenic cats should be weighed carefully against potential risks of increased virus replication or induction of neutropenia. See Chapter 2 and the Drug Formulary in the Appendix, for further information.

Treatment of FIV-associated stomatitis is often difficult. Repeated treatment with dental cleaning and antibacterials may offer palliative relief but is rarely sufficient to resolve the lesions. Although the pathogenesis of stomatitis is considered to be immune-mediated, glucocorticoids should be avoided in FIV-associated stomatitis. In some cases, treatment with AZT can be beneficial (see Table 12-3). Topical bovine lactoferrin has also been beneficial for FIV-related stomatitis (see also Chapter 2 and the Drug Formulary in the Appendix).[903] Lactoferrin, a glycoprotein present in exocrine secretions and neutrophils, plays an important role in the host defense system, and its anti-inflammatory effect in the stomatitis of FIV-infected cats has been attributed in part to its involvement in the regulation of neutrophil function. Available evidence suggests that bovine lactoferrin may have the potential to improve and protect against consequences of overactivated lymphocytes by modulating cell proliferation, cell cycle, and cytokine expression in FIV-infected cats.[528] If these medications are not helpful in severe cases, an effective treatment can be extraction of all teeth (Fig. 12-4), paying careful attention to removal of all of the roots of the teeth. In many cases, long-term resolution of inflammation is achieved by this treatment, and cats return to eating a normal diet.

In some cats with FIV-related neurologic disorders, AZT treatment has been beneficial, whereas some cats require glucocorticoid therapy to help reduce cerebral inflammation. Glucocorticoids have been used in acute and chronic experimental FIV infections. In acute infections, increases in plasma viremia and decreased CD8+ cell activity were noted, while beneficial effects have included delays in the onset of brainstem auditory-evoked potentials abnormalities, or their normalization in chronically infected cats.[52] Because of the effects on viremia, indiscriminate treatment with glucocorticoids or other immunosuppressive drugs should be avoided unless a compelling indication for their use exists.[14,585]

Intact male and female FIV-infected cats should be neutered to reduce stress associated with estrus and mating behavior and the desire to roam outside the house or interact aggressively with other cats. Surgery is generally well tolerated by asymptomatic FIV-infected cats, although perioperative antibacterial administration should be considered. Because the virus survives only minutes outside the host and is inactivated by all disinfectants, including common soap, simple precautions and routine cleaning procedures will prevent transmission while in the hospital. FIV-infected patients should be kept in individual cages during hospitalization.[14,584,585,858]

Opinions about general vaccination of FIV-infected cats differ. Experimental evidence shows that FIV-infected cats are able to mount immune responses to administered antigens,[198,562] but responses may be impaired during the terminal phase of infection.[308] FIV-infected cats have developed illness after receiving modified live virus feline panleukopenia vaccine (see Postvaccinal Complications, Systemic Illness, Chapter 100), so inactivated vaccines should be considered.[861] There is debate about the negative effect of vaccine-induced immunostimulation in FIV-infected cats. Some studies suggest that immune stimulation may help stabilize CD4+ cell numbers.[855] In contrast, stimulation of FIV-infected lymphocytes is also known to promote virus production in vitro. In vivo, vaccination of chronically infected FIV-infected cats with a synthetic peptide was associated with a

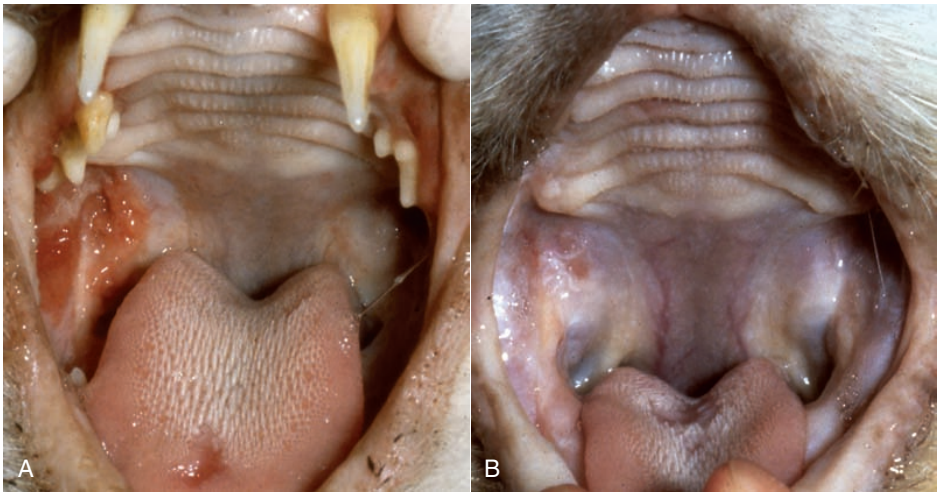

FIG. 12-4 A, Stomatitis in the glossopharyngeal fauces in an FIV-infected cat before dental extraction. **B,** Stomatitis with less inflammation following dental extraction from the cat in *A.* (**A**, Courtesy Julie Levy, University of Florida, Gainesville, FL.)

decrease in the CD4/CD8 ratio.[573] As described earlier, lymphocyte stimulation can increase the expression of cellular FIV receptors and increase virus production, a combination that could enhance progression of infection. Thus, vaccination and antigenic stimulation may potentially be disadvantageous with a potential tradeoff of protection from infection for progression of FIV infection secondary to increased virus production, although this has not been proven in experimental studies. If adult FIV-infected cats are kept strictly indoors, the risk of being infected with other pathogens may be lower than the possible harmful effect of vaccination. If potential exposure to parvovirus, herpesvirus, or calicivirus cannot be excluded, only core vaccines (against panleukopenia and upper respiratory infection) and, when available, inactivated vaccines should be considered.

PREVENTION

Identification and segregation of infected cats is considered the most effective method for preventing new infections in other cats. Nonetheless, because of its utility as a model for HIV, there has been an enormous amount of work invested in FIV vaccine development. There have been few successes despite a large number of different approaches taken in attempts to create FIV vaccines,* including DNA and subunit vaccines with or without adjuvants such as cytokines,† peptide or recombinant vector vaccines using various elements of the FIV or HIV virion,‡ mutant viruses,[108,530,619,801] fixed or unfixed infected cells,[320,851] or inactivated or attenuated virus.§ Various routes of vaccine administration, including mucosal application and injection directly into a lymph node, have also been explored.[290,291,565,985] One result of this work has been the introduction of an FIV vaccine licensed in the United States (see Web Appendix 3). The marketing of this vaccine has been met with controversy regarding its use, the interpretation of commercially available FIV tests, and the extent of protection afforded by the vaccine.[171,406,495,699] Using available antibody tests including immunoblotting (Western blots), antibodies detected after vaccination cannot be distinguished from those caused by natural infection.[363] Another concern with FIV vaccines is theoretical enhancement of infection manifest by increased viral loads and/or accelerated development of viremia. Vaccine enhancement has been seen in vitro and in vivo with peptide and recombinant vaccines, subunit vaccines, and mutant virus vaccines.* The upregulation of CD134 with lymphocyte activation, as would happen after vaccination, may account for some of the elements of vaccine enhancement, because such events would increase the number of cells susceptible to FIV infection.[254,449]

A major obstacle in design of a widely effective vaccine against FIV infection is the large genetic diversity among viral isolates. Sequence divergence within a subtype ranges from 2% to 15%, and that between subtypes ranges up to 26%. One study[893] has suggested that as little as a two-amino-acids change in a part of an envelope protein could result in immune evasion, further emphasizing the hurdles posed by genetic diversity of the virus. Single-strain vaccines have only provided adequate protection against homologous and closely related strains, but not against moderately to greatly heterologous strains. The vaccine licensed in the United States is a dual-subtype vaccine containing inactivated FIV subtype A and subtype D with an adjuvant.[842,1033] The combination of two genetically distinct subtypes elicits strong anti-FIV cellular immunity[751] and broad-spectrum virus-neutralizing antibodies. This vaccine has not been field-tested against natural FIV infection in controlled studies. In experimental conditions, it has shown some ability to protect against B subtype viruses[441,442,543,841] frequently found in the United States. In some cats, protection from challenge infection after vaccination is afforded for up to 48 weeks.[441] Given the challenges[424,425] of developing widely effective vaccines, results of some work[644] suggest that a goal of vaccination could be preservation of immune function or disease prevention rather than prevention of infection. Because the vaccine contains whole virus, cats respond to vaccination by producing antibodies that are indistinguishable from those produced during natural infection.[1033]

The existing literature on FIV vaccines gives some reasons for scrutiny when using any FIV vaccine. First, although many vaccine studies have shown protection against FIV infection after challenge

*References 269, 355, 804, 1011, 1057.
†References 185, 256, 304, 370, 416, 448, 450, 451, 453, 802.
‡References 96, 133, 168, 184, 290, 300, 317, 576, 577, 863, 1001, 1010.
§References 254, 291, 344, 415, 427, 433, 508, 209, 664, 666, 667, 805, 805.

*References 73, 343, 450, 526, 799, 800.

with either homologous or, in some instances, heterologous isolates, the results have been quite inconsistent. In a particularly telling study, cats vaccinated with inactivated whole virus were not protected against challenge with a heterologous isolate even though the isolates used in the study were within the same subtype (subtype A) as the vaccine isolate.[427] The isolates of this study were all of different pathogenicity, emphasizing the importance of understanding the challenge inocula and the vaccine strategy employed. The difficulties in developing FIV vaccines need to be understood by the clinician when evaluating vaccine claims, and for FIV, perhaps more facets need to be considered than with other infectious agents (Web Box 12-1).[424,425] Although not demonstrated with the commercially available vaccine, in some instances, concern still exists that vaccination against FIV may actually enhance infection.[344,508,509,862] For all the reasons previously noted, the best prevention of FIV infection remains segregation of FIV-infected from noninfected cats.[3]

Other strategies for preventing infection have been explored. Protection against homologous isolates has been achieved by passive immunization and adoptive transfer of lymphocytes from vaccinated cats.[844] Kittens may be protected from infection if the queen has a high concentration of FIV-specific antibody, suggesting that stimulation of humoral immunity has a role in protection.[417,843] Detection of cytotoxic lymphocyte activity after vaccination implicates a role for CMI in protection.[305,844,1002]

PUBLIC HEALTH AND OTHER CONSIDERATIONS

FIV is a feline pathogen, and no demonstrated evidence has been found that it can infect humans, even those such as veterinarians who are at greater risk of exposure.[137] However, infection of human peripheral blood mononuclear cells has been accomplished in vitro with a laboratory-maintained FIV isolate,[482,485] and clinical disease was induced in cynomolgus monkeys after autologous transfusion with peripheral blood mononuclear cells infected in vitro.[481] However, no evidence has been found to link FIV infection to any human disease, including AIDS. Investigations have failed to identify antibodies in humans who have been bitten by infected cats or who have inadvertently injected themselves with virus-containing material.[1106]

A large number of studies have documented the potential for FIV to serve as a gene-delivery system with the ultimate goal of treating nonlentiviral diseases. Genetically manipulated isolates of FIV have shown some promise as a vector for gene transfer in a number of systems involving cell lines derived from humans and other species, and for the treatment of mouse models of human disease (e.g., hemophilia).*

*References 55, 56, 160, 283, 374, 471, 500, 521, 546, 547, 550, 918, 934, 943, 944, 946, 1043.

CHAPTER 13

Feline Adenovirus Infection

Frances A. Kennedy

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■ References ■ Images ■ Web Tables, Boxes, and Appendices

Clinically apparent disease caused by primary adenoviral infection is most common in immunologically compromised animals.[1,2] In studies of cats in Hungary, Scotland, the Netherlands, the Czech Republic, and the United States, serologic findings indicated adenovirus exposure in 15%, 10%, 20%, 25%, and 26% of cats, respectively.[6,7,10] However, only one case of confirmed disseminated adenovirus infection in a cat has been reported.[4,5] Inclusion body hepatitis reported in a black panther[3] was suggestive of adenovirus infection; however, the causative agent could not be confirmed by electron microscopy (EM) or by virologic identification.

In the confirmed case of disseminated adenovirus infection, a comatose, 8-year-old, spayed female, domestic shorthair cat had petechiae on the oral mucous membranes. Abnormal hematologic findings included leukopenia (2100/μL) and thrombocytopenia (73,000/μL). Treatment with intravenous lactated Ringer's solution, dexamethasone, and vitamin K produced no response. The cat died 4 hours after presentation.

At necropsy, the abdominal cavity and pericardial sac were filled with serous fluid. Serosal and mucosal surfaces of the small and large intestines were diffusely dark red with scattered serosal petechiae, and the intestinal contents were fluid and dark red. The liver and kidneys were swollen, and the liver had an accentuated lobular pattern.

An undiluted sample of serous abdominal fluid was positive for the group-specific antigen (p27) of the feline leukemia virus (FeLV) and was negative for antibody to the feline immunodeficiency virus. A specimen of ileum was positive for feline coronavirus by direct fluorescent antibody testing. Result of an enzyme-linked immunosorbent assay for feline panleukopenia virus was negative on specimens of liver, kidney, ileum, mesenteric lymph node, and spleen. An adenovirus particle was identified by EM examination of the intestinal contents.

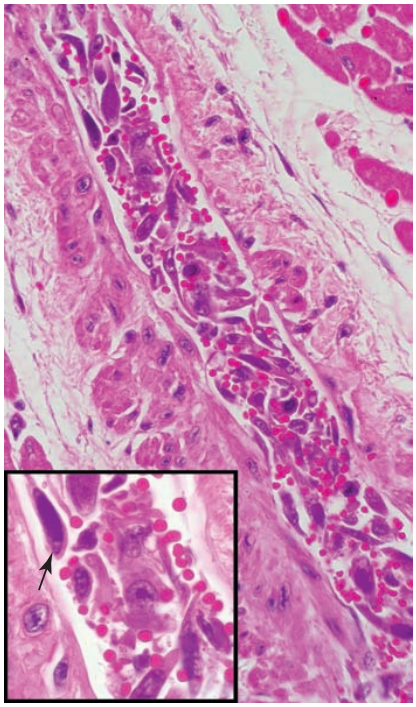

FIG. 13-1 Photomicrograph of an intramyocardial artery with sloughed endothelial cells. Spindle-shape cells within the arterial lumen have large, pleomorphic nuclei, some with inclusion bodies (H&E stain, ×400). **Inset,** Nucleus containing an inclusion body (*arrow*; H&E stain, ×1000). (Courtesy Frances A. Kennedy, Michigan State University, East Lansing, MI.)

Histologically, endothelial cells were detached from intramyocardial arteries. These sloughed cells were large, spindle shaped, and occasionally multinucleated (Fig. 13-1). Nuclei of sloughed cells were large and pleomorphic, and many contained intranuclear inclusion bodies. Multiple round eosinophilic inclusions were present in some nuclei, with amphophilic granular inclusions filling other nuclei. Some nuclei were almost filled with well-delineated basophilic inclusions, with margination of the small amount of surrounding chromatin (Fig. 13-1, *Inset*). Some of these latter nuclei had indistinct borders, resulting in a "smudge cell" appearance. Cytoplasm of sloughed endothelial cells was eosinophilic. Minimal perivascular infiltrates of lymphocytes were present in the myocardium.

The stomach had diffuse submucosal edema. Diffuse superficial necrotizing and hemorrhagic enteritis with submucosal edema was present in the small intestine. Necrosis was more severe in the ileum, with full-thickness mucosal necrosis over Peyer's patches. There was moderate lymphoid depletion and peripheral hemorrhage in submucosal lymphoid tissue. Sections of colon were comparably affected, with submucosal edema and particularly severe mucosal necrosis overlying areas of submucosal lymphoid tissue. Submucosal and mesenteric blood vessels at all levels of the gastrointestinal tract had endothelial lesions as described in the heart. Similar vascular lesions were seen in small hepatic arteries, pulmonary arteries, trachea, thymic remnant, urinary bladder, thyroid gland, adrenal gland, bone marrow, spleen, lymph node and kidney. There was depletion of lymphoid tissue in the spleen and lymph nodes.

EM examination of detached endothelial cells revealed intranuclear viral particles measuring approximately 65 nm in diameter (Fig. 13-2). Some of these particles were roughly icosahedral, with dense central cores. In some areas, viral particles formed loose crystalline arrays.

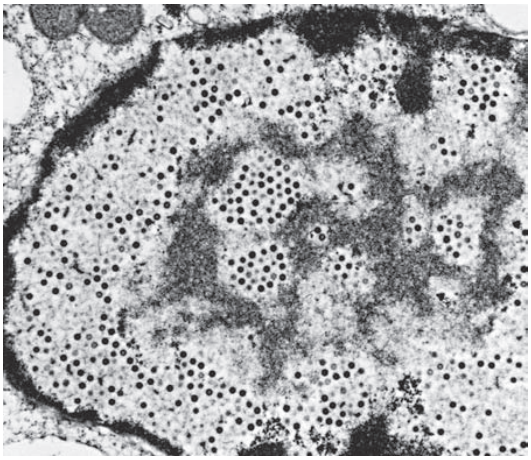

FIG. 13-2 Electron micrograph of an endothelial cell with intranuclear viral particles. Moderate autolytic change is responsible for disruption of adenoviral arrays (×17,900). (From Kennedy FA, Mullaney TP. 1993. *J Vet Diagn Invest* 5:273-276.)

Because adenoviruses are considered to be highly host specific,[1] the clinically healthy horses, dogs, and one goat on the property were unlikely sources of infection for this cat. Other cats on the property were similarly clinically healthy. It is possible that FeLV infection produced an immunodeficient state in the affected cat, predisposing it to develop disseminated adenovirus infection. Leukopenia may have been a consequence of FeLV infection, terminal endotoxemia, or both. Thrombocytopenia was most likely consequent to consumption secondary to vascular lesions, because there were adequate numbers of megakaryocytes in the bone marrow specimen. The positive direct fluorescent antibody test for feline coronavirus on the ileum was likely indicative of a subclinical infection with feline enteric coronavirus. No gross or histologic lesions typical of feline infectious peritonitis were found.

Results of serologic studies in cats, measuring serum antibody to feline adenovirus, have indicated that persistent subclinical adenovirus infection exists in the feline population. Significant increases in rates of positive titer results have been found with increasing age and among cats with respiratory or gastrointestinal signs.[10] However, to date, only one case of adenovirus infection in a cat has been confirmed by the polymerase chain reaction.[8,9] The affected cat had a period of transient hepatic failure, and serologic testing confirmed adenovirus infection. Adenovirus hexon capsid nucleic acid was detected in two rectal swabs taken at a 1-year interval and a pharyngeal swab taken at the second sampling from this cat. This finding suggests that, in addition to persistent subclinical infections, cats may be a source of persistent adenovirus shedding.

In a case of gastroenteritis in a 1-year-old female child, a fecal specimen was positive for an adenovirus strain that was highly homologous with feline adenovirus.[12] No cats were kept in this household; however, this infection was interpreted as suggesting interspecies transmission of adenovirus between felines and humans. Cross-species adaptability of adenovirus has been suggested by the finding of a feline adenovirus isolate that could replicate in both monkey Vero cells and human HeLa cells.[11]