Pathologic Features of Acquired Immunodeficiency-Like Syndrome in Cats Experimentally Infected with Feline Immunodeficiency Virus

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ABSTRACT. Five specific pathogen free cats were inoculated with feline immunodeficiency virus (FIV) isolated in Japan to observe changes toward development of acquired immunodeficiency syndrome (AIDS)-like disease. All inoculated cats had lymphadenopathy and mild respiratory disease shortly after inoculation. Following the initial acute phase lasting for more than 40 weeks, the clinical signs gradually diminished in three animals, and the asymptomatic carrier (AC) stage was observed at 45 (1 cat) and 70 (2 cats) weeks postinoculation (p.i.). Two of the three cats developed respiratory signs and diarrhea at 105 or 106 weeks p.i. One cat died at 121 weeks p.i. with severe wasting, with necropsy findings consistent with AIDS-related complex (ARC). The others were surviving at 150 weeks p.i. with mild clinical signs or asymptomatic. Another group of two cats developed more severe illness without the AC phase. One died at 48 weeks p.i. with the ARC illness. The other cat developed marked emaciation with diarrhea at 75 weeks p.i., and died at 108 weeks p.i. with a histologic diagnosis suggestive of terminal immunodeficiency. Histologically, the lymph nodes showed serial changes toward the terminal illness, from follicular hyperplasia at the acute phase to the lymphoid depletion at the ARC and AIDS-like terminal stages. The FIV antigen was demonstrated in the lymph nodes. The virus was isolated from peripheral blood mononuclear cells of all the inoculated animals. These data demonstrated possible etiologic association of FIV with development of AIDS-like disorders in the cat.—KEY WORDS: AIDS, feline, feline immunodeficiency virus, retrovirus.


Feline immunodeficiency virus (FIV) was first isolated in a cluster of cats with an acquired immunodeficiency (AIDS)-like disease [37]. The subsequent worldwide serologic surveys have led to identifications of a great number of naturally infected animals, both symptomatic and asymptomatic [5, 9, 17, 18, 21, 26, 44, 54, 56]. Clinical and pathologic observations on these infected cats have confirmed the initial observations made by Pedersen et al. [37], and AIDS-like disorders have been closely associated with FIV infection [13, 17, 18, 21, 23, 27, 44, 48, 54, 56].

The clinical stages include the five stages reported with human immunodeficiency virus (HIV) infection [12, 25], and a long asymptomatic carrier (AC) stage is considered to precede the symptomatic AIDS-related complex (ARC) and AIDS stages. Furthermore, immunologic evaluations of both experimentally and naturally infected cats have demonstrated presence of immunodeficiency with impaired T-cell functions [1, 3, 28, 45, 49–51]. A morphologic evidence of development of immunodeficiency has been provided by the lymph node pathology similar to those in human AIDS patients [7].

Thus, the accumulated knowledge strongly indicated etiologic association of FIV with the feline AIDS-like disorder. This assumption, however, remains to be verified by experimental facts. The experimental infection has not previously been successful in induction of AIDS-like diseases. The acute phase illness and progression into the AC stage have been reported by a number of researchers [1, 3, 28, 36, 37, 51, 57]. The failure to induce the experimental disease in FIV infected cats so far may be related to the virus strain, time required for development of clinical syndromes, or requirement for cofactors. In order to challenge the first possible problem, we employed an isolate in Japan, where a greater number of clinical FIV cases have been experienced [26, 27]. This report presents the results of the experimental FIV infection where disease progression and mortality were recorded in multiple cases.

MATERIALS AND METHODS

Animals: Five specific pathogen free (SPF) American domestic short hair cats were used for the inoculation study (Table 1). The cats were originally derived from Dr. N. C. Pedersen, University of California, Davis, U.S.A., and were bred in this laboratory for the study. They were negative for antibodies to FIV, or feline infectious peritonitis virus (FIPV) and were feline leukemia virus (FeLV) p27-negative. Another group of three SPF cats were maintained in the same environment as uninfected controls. During the experimental infection, the cats were housed in individual cages in a semi-closed environment, and were fed commercial diet ad libitum (Kal Kan, Master Foods Ltd., Tokyo Japan).

FIV antibody detection: The serum antibodies to FIV were assayed by indirect immunofluorescence (IFA) with infected lymphocytes, and by Western blot with purified FIV antigen as described previously [21].

Virus: The FIV-GA3 strain originally isolated at National Institute of Health (Chiba, J. and Nagata, S., manuscript in preparation) from a Japanese domestic cat
showing an AIDS-like illness was used for infection. The virus was grown in an interleukin-2 (IL-2)-dependent feline T-lymphoblastoid cell line of a SPF cat origin (KTC) (Chiba, J. and Nagata, S., manuscript in preparation). The virus preparation did not contain any detectable level of FeLV p27 by a double sandwich ELISA using two mouse monoclonal antibodies to p27 prepared by the authors [29]. The culture fluid initially exhibiting a Mg2+-dependent reverse transcriptase activity of 30,000 cpm was concentrated ten-fold, and 0.2 ml of this material, mixed with the same volume of sterile phosphate buffered saline (PBS), was inoculated intramuscularly into the cats. The uninfected cats received no inoculation, but were maintained in the individual cages placed in the same room.

Virus isolation: The peripheral blood mononuclear cells (PBMC) from infected cats were cocultivated with uninfected SPF cat PBMC under stimulation with concanavalin A (Con A) and IL-2 as previously described [37]. The virus antigen in the culture was identified by IFA using an anti-FIV p24 monoclonal antibody (supplied by Dr. N. C. Pedersen, University of California, Davis).

Biopsy: One popliteal lymph node from each animal was biopsied either at 17, 27 or 45 weeks after inoculation.

Histopathology: Tissues were fixed in 10% neutral buffered formalin and paraffin embedded, and the sections routinely stained with hematoxylin and eosin (H&E). The FIV antigen was demonstrated by using the anti-p24 monoclonal antibody with an indirect immunoperoxidase method on paraffin sections.

RESULTS

Clinical course: The clinical outcome of each inoculated animal is summarized in Table 1. Anti-p24 antibody that recognizes the core protein of FIV was detected from sera of all five FIV-inoculated cats at 4 weeks after exposure and persisted thereafter. Anti-envelope gp120 antibody first appeared at 3 weeks to 8 weeks postinoculation (p.i.).

Following inoculation, detectable lymphadenopathy developed with the day of onset ranging between 16 days (No. 2) and 49 days (No. 3) p.i. Transient fever was seen in three cats (Nos. 1, 4 and 5) with signs of mild intermittent upper respiratory diseases (sneezing, cough, and nasal discharge), appearing at 16 days (No. 2) to 57 days (No. 1) p.i. Cyclic neutropenia was observed in all animals beginning at 35 days p.i., and persisted until 15 to 20 weeks p.i. This initial asymptomatic phase persisted in all five cats for more than 40 weeks. In three animals (Nos. 1, 3 and 4), the clinical signs then gradually diminished, and the AC stage was observed at 45 (No. 1) and 70 (Nos. 3 and 4) weeks p.i.

The cat No. 3, with a distinct asymptomatic period between 70 and 104 weeks p.i., developed wasting with severe respiratory disease at 105 weeks p.i. The cat died at 121 weeks with marked emaciation. The cat No. 4 developed diarrhea and respiratory signs at 106 weeks, but was still surviving with mild clinical signs at 150 weeks p.i.

The two cats (Nos. 2 and 5) developed more severe clinical signs instead of becoming asymptomatic. The cat No. 5 showed mild lymphadenopathy and upper respiratory signs up to 40 weeks p.i., then developed severe diarrhea, emaciation and dehydration, and died at 48 weeks p.i. The cat No. 2 had lymphadenopathy and persistent upper respiratory disease as the acute phase illness, and then developed protracted diarrhea at 60 weeks. The disease was persistent and progressive, and marked emaciation with diarrhea was noted at 75 weeks p.i. The cat died of respiratory failure at 100 weeks p.i. There was no evidence of disease or mortality in three uninfected control cats during this experiment.

Lymph node biopsy: One popliteal node from each infected animal was biopsied at 17 to 45 weeks p.i. in order to serially observe the initial histologic changes (Table 2). All the nodes biopsied were enlarged (largest
1.4 × 0.9 × 0.4 cm, Cat No. 3 at 45 weeks p.i.). Histologically, all the lymph nodes had an increased number of follicles and contained prominent germinal centers with tingible body macrophages and mitotic figures (Fig. 1). The sinus contained numerous histiocytes and lymphocytes.

The p24 antigen was found in all lymph nodes examined. The antigen positive cells were most often located in the medullary sinus area and were mainly histiocytes or macrophages. In addition, a small number of lymphocyte-like cells and tingible body macrophages in the germinal centers and interfollicular cells were stained for the p24. The antigen staining was more intense in the nodes biopsied earlier than in those obtained later.

Necropsy: In cat No. 2, gross findings included wasting (body weight 1.7 kg), severe dehydration, poor hair coat and skeletal muscle atrophy. Edema was seen in the lung. The trachea and bronchi contained mucopurulent exudate. The liver slightly decreased in size with an enhanced reticular pattern. The kidneys showed irregular surface. The lymph nodes decreased in size.

Both No. 3 and No. 5 cats showed marked dehydration and emaciation with poor hair coat, but the lymph nodes retained their normal size if not enlarged. The No. 3 cat had the enlarged left kidney and atrophic right kidney.

On microscopic examination, the lung of No. 2 cat revealed bacterial bronchopneumonia with degenerated neutrophils and macrophages (Fig. 2). The liver showed a pattern of centrilobular hepatic atrophy and mild neutrophilic cholangiohepatitis. The kidney showed mild interstitial nephritis with tubular calcification. Extramedullary hematopoiesis and follicular depletion were observed in the spleen. The axillary, popliteal, bronchopulmonary, anterior mediastinal, and mesenteric lymph nodes showed severe lymphoid depletion and sinus histiocytosis consistent with those seen in human AIDS (Fig. 3). The germinal centers were lost and the number of cortical lymphocytes was markedly decreased. The mandibular lymph nodes showed depletion with hyalinization of germinal centers. Immunohistochemistry with the anti-p24 antibody revealed more medullary sinus cells positively stained than in the biopsy specimens obtained in the earlier phase of the disease. These large round sinus cells with p24 staining had a morphology consistent with macrophages (Fig. 4). In addition to the sinus cell staining, small numbers of lymphocyte-like cells in paracortical and cortical areas were seen with positive p24 staining. The bone marrow showed a prominent granulopoiesis with decreased erythropoiesis, with a myeloid/erythroid ratio of approximately 10 (Fig. 5). There was no histologic evidence of encephalitis.

In cat No. 3, microscopic examination of the lung revealed pneumonia characterized by alveolar purulent exudate. In the liver, mononuclear cell infiltration in the Glisson's capsule was seen. The kidney showed moderate interstitial nephritis with glomerulonephritis. The spleen and mandibular lymph nodes had lymphoid follicles with obscure margins, and some follicles had partially hyalinized germinal centers. The axillary, inguinal, and mesenteric lymph nodes lacked prominent follicles with in-
Fig. 3. Lymphoid depletion in the popliteal node (Cat No. 2). The cortical area is markedly reduced. Follicles are inapparent. HE stain, × 40.

Fig. 4. Localization of FIV p24 antigen in the bronchopulmonary node (Cat No. 2). Darkly stained positive cells with a macrophage-like morphology are seen in the medullary sinus. × 200.

Fig. 5. Hyperplastic bone marrow with myeloid hyperplasia (Cat No. 2). HE stain, × 200.

Fig. 6. Small atrophic germinal centers in the popliteal lymph node of Cat No. 5 at necropsy. There is a decrease in the activity of the germinal centers. HE stain, × 40.
creased fibrous components. Immunohistochemical staining revealed the FIV antigen with an identical pattern with Cat No. 2. The gastric and duodenal mucosa were partially infiltrated with mononuclear cells, and atrophy of mucosa was noted. The urinary bladder showed hemorrhagic erosion of the mucosa and degenerated neutrophils and transitional epithelial cells were seen in the lumen.

In Cat No. 5, the lung showed mild bronchopneumonia with purulent and mononuclear cell infiltration and the serous gland hyperplasia around the bronchi and bronchioles. Proliferation of the glomerular mesangial cells was seen in the kidney. Mild follicular depletion and hyalinization of germinal centers were seen in the spleen. The lymph nodes showed a mixed lesion of follicular hyperplasia and degeneration, and some centers were in the process of atrophy with hyalinization (Fig. 6). The small intestinal mucosa was infiltrated with inflammatory cells (lymphocytes, plasma cells and granulocytes). The follicles were uniformly enlarged, but the shape was irregular and some germinal centers showed decreased cellularity with fibrosis. The interfollicular tissue of the poliptical node and medullary sinus of the axillary and mesenteric nodes also had increased fibrous components. A prominent staining of the sinus area macrophages with the anti-p24 antibody was observed. In addition to the sinus staining, small numbers of solitary cells in the follicles were also stained.

Virus isolation: The virus was reisolated from the PBMC culture of all the dead animals as well as the remaining two animals. The viruses were all identified as FIV by immunoblotting with specific anti-FIV antibodies.

DISCUSSION

A clinical disease syndrome mimicking the natural FIV infection was successfully produced in the present study by inoculating five SPF cats with a Japanese isolate of FIV. The overall mortality at 150 weeks p.i. was 60% (3/5). The FIV infection was confirmed by the continuous presence of antibodies to FIV proteins after a short incubation period, presence of FIV p24 antigen in the lymph nodes, and by virus isolation made at the time of death or at the end of the experiment.

Clinical features of the initial phase of HIV infection include generalized lymphadenopathy associated with seroconversion and it is often described as a mononucleosis-like syndrome. Following resolution of the primary disease, the infection merges into the asymptomatic phase. After a long period of 3 to 10 years, ARC develops from an asymptomatic infection to constitutional symptoms of fever (persisting more than 1 month), weight loss (greater than 10% of baseline), diarrhea (persisting more than 1 month) or neurologic disease accompanied by persistent generalized lymphadenopathy (PGL). Finally, opportunistic infections and/or neoplasms are seen in the AIDS stage [2, 34, 35, 40, 53]. This clinical progression is paralleled in lymph nodes, lesions beginning with follicular hyperplasia in the early stages and ending in lymphocyte depletion. Biopsy studies of patients with lymphadenopathy have shown predominantly hyperplastic lymph nodes [11, 19, 24, 31]. By the stages of ARC or AIDS, hyperplasia has often regressed, often with hyalinized or absent germinal centers. In the patients with opportunistic infections, lymphoid depletion with abundant proliferation of fibrous tissue was predominant [8, 11, 14, 30, 35, 39]. These findings, taken together with the clinical findings, indicate that histologic evidence of severe loss of lymphoid tissue mirrored the loss of immunologic function in AIDS patients [6, 10, 43].

The first detectable clinical sign in cats inoculated with FIV was lymphadenopathy starting at 16 to 49 days p.i., while antibodies to FIV developed at 4 weeks p.i. in all animals. Although the appearance of FIV antibodies and lymphadenopathy did not strictly correlate with each other, these two early events were characteristic of the acute phase as described earlier [47, 57]. Also other signs that followed were in good agreement with other experimental FIV infection systems [37, 38, 57], and the acute phase illnesses were similar to those in HIV infection. Although two animals with relatively short incubation died (No. 2, 16 days; No. 5, 25 days), the animal with the longest incubation period also died (No. 3, 49 days). Therefore, the incubation period may not be a determinant factor for prognosis.

Disappearance of the clinical signs then was considered to be the start of the AC phase [25]. Three animals showed a distinct asymptomatic phase. The other two animals, however, developed more severe illnesses without any asymptomatic period. The severe clinical signs seen in these cats were characteristic to those reported earlier as ARC [25]. Total of four animals went into the ARC phase, and three died at 48, 100 and 121 weeks p.i. There was no morbidity or mortality, however, in uninfected control animals maintained in the same environment.

Necropsy revealed pneumonia in all cases, but the one (No. 5) that died rather acutely at 48 weeks p.i. had the mildest pulmonary lesions. The other two animals had severe bacterial infection of the lung which can be interpreted as an opportunistic infection. They had no intranuclear or cytoplasmic inclusion bodies, or interstitial pneumonia, and these lesions were not attributed for feline viral rhinotracheitis virus or feline calicivirus infections. Although the naturally infected cats died of terminal AIDS illnesses invariably had more severe opportunistic infections such as cryptococcosis, toxoplasmosis, hemobartonellosis or atypical mycobacteriosis [15, 17, 22, 23, 26, 38, 54], it may not be possible to see these infections in a semi-closed experimental setting.

Although lymph node pathology has been reported to be less specific in diagnosing lentivirus-associated immunodeficiency [41], it is still useful for following the disease stages of cats with known FIV infection as in HIV infected individuals. The cat No. 2 had the most severe lymphoid depletion of all nodes examined. Therefore, the lymph node pathology in conjunction with the evidence
for pulmonary infection of an opportunistic nature supported a possible diagnosis of AIDS. The cat No. 3, on the other hand, was considered to have died at the terminal ARC phase with the lymph node showing only partial depletion. The degree of follicular changes varied from one node to another, and it was interpreted as being in the process of degenerative changes toward the terminal immunodeficiency lymph node pathology. The cat No. 5 which died acutely had mixed lesions of hyperplasia and degeneration. Since the acute phase node is characterized by solely follicular hyperplasia, this histopathologic change was interpreted as that in the ARC phase where morphologic evidences of immunodeficiency appear.

On the basis of these clinical and pathologic findings, it is possible that the cat No. 2 died of an AIDS-like disease at 100 weeks p.i., following 40 weeks of ARC and AIDS-like phases. Although a clear distinction between the two phases is not possible, the AIDS-like phase may have started at 75 weeks p.i. when marked emaciation was first noted.

Findings from the lymph node biopsy and necropsy gave an insight into the serial pathologic changes of the lymphoid organ toward development of the AIDS-like disease as discussed with HIV patients [14, 39]. The initial lesion of follicular hyperplasia was seen during the acute phase. The lymphadenopathy was not marked during the subsequent asymptomatic phase, and the hyperplasia may have diminished temporarily. In the ARC phase, however, the hyperplasia was again noted, and a degenerative process developed concurrently with the ongoing follicular hyperplasia, which is in good agreement with the pathologic changes in HIV infected individuals [33]. The initial degenerative changes included obscure follicular margin, follicular atrophy and segmentation. Hyalinization of the germinal centers was also seen. The final stage lesions included disappearance of the follicular and paracortical lymphocytes and development of fibrosis. These findings revealed that the morphologic alterations of lymph nodes were essentially similar to those in HIV infection.

The FIV antigen seen in the follicular lymphocytes in the acute phase may be closely related to the development of follicular hyperplasia and subsequent degenerative changes. The antigen bearing lymphocytes later decreased in number, and the antigen was mainly detected in sinus macrophages. All these findings are compatible with the observations in human HIV patients [4, 20, 52] and monkeys infected with simian immunodeficiency viruses [32, 42, 46, 52, 55]. These findings suggest that macrophages also play a role in the pathogenesis of FIV-induced lymph node lesions. It has been shown that the virus production by HIV-infected macrophages was high and long-lived with a possible role of virus dissemination and persistence [16].

The disease progression observed in this study almost paralleled those previously reported with natural FIV infection [25]. The lack of the AC phase may be due to more severe course of infection relative to natural infection. The virus strain and/or the large dose of virus inoculated might explain the rapid disease progression.

Experimental infection of cats with Petaluma isolate has not reproduced AIDS in spite of vigorous efforts worldwide. Since we have used almost identical cats as Pedersen and his colleagues have been using, the determinant factor for producing clinical diseases might be virulence of the virus we have used. It will be necessary to compare the biologic properties and genetic structure of GA-3 strain with other FIV isolates.

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