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Primary Stage of Feline Immunodeficiency Virus Infection: Viral Dissemination and Cellular Targets

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The objective of this study was to identify cellular and organ targets of acute feline immunodeficiency virus (FIV) infection in vivo. Tissues of FIV-infected cats were studied at eight time points during the first 3 months after experimental infection. FIV nucleic acids were first detected by in situ hybridization 21 days after infection, approximately 1.5 weeks after lymph node enlargement was first observed and 3 weeks before the primary acute flu-like illness. The majority of FIV-infected cells were present in lymphoid organs, though low numbers of infected cells were noted in nonlymphoid organs as well. Germinal centers harbored many of the FIV-infected cells within lymphoid tissues. The thymic cortex was also a major site of early infection. Combined in situ hybridization and immunohistochemistry revealed that T lymphocytes were the primary target of early FIV infection in tissues of cats before the onset of clinical signs of acute illness. An unidentified population of mononuclear cells and a few macrophages were also infected. During the ensuing acute flu-like illness, the proportion of FIV-infected macrophages in tissues increased dramatically. This early shift in the predominant cellular localization of FIV from T lymphocytes to macrophages may be important for establishing viral persistence.

The human, simian, and feline immunodeficiency viruses (HIV, SIV, and FIV, respectively) are lentiviruses that share a common disease pattern featuring a primary acute flu-like illness shortly after infection, followed by an asymptomatic stage of variable length, and terminating with complications associated with severe immunodeficiency. The majority of research on these viruses has focused on the terminal AIDS stage of infection. However, many important processes occur in the primary acute stage of infection that may influence the subsequent disease course (22, 42, 53, 67). The decline in $CD4^+/CD8^+$ T-lymphocyte ratio characteristic of lentivirus-induced immunodeficiency disease is first evident early after infection (2, 29, 45). HIV viral burden is high with much active replication of virus during the acute primary stage of disease (17, 19, 28, 76). Viral variants are generated rapidly during this period of elevated replication and may be important in subsequent disease pathogenesis (53). Early virus dissemination may also be important for determining the subsequent course of disease. This replication and spread of virus in the acute stage of infection is brought under control by the host immune response. The strength of the primary immune response to immunodeficiency virus infection has been correlated to survival time (22, 67). Which branch of the immune system is preferentially activated early after infection may also be important in determining disease outcome (18). Recent evidence suggests that progression to AIDS may be associated with a T_H2-type response, while resistance may be higher in individuals with a strong T_H1-type response (18). Therefore, understanding of the immunologic and virologic properties of the very early stages of HIV infection will be important for understanding disease pathogenesis and designing interventive therapies. The very early events of HIV infection are difficult to study, however, because HIV-infected individuals are normally unaware of their infection status until later in the disease course. Animal models will therefore be an important tool for examining the primary stage of immunodeficiency-causing lentivirus infections.

Experimentally FIV-infected cats have a well-defined transient acute flu-like illness followed by recovery and an extended asymptomatic stage (38, 56, 57, 70). $CD4^+/CD8^+$ T lymphocyte ratios decline rapidly during the primary acute illness and continue to drop slowly throughout the disease course. This model is therefore well suited for studying early events of immunodeficiency virus infection and the mechanisms of containment of early viral replication. Viral dissemination and cell tropism of FIV and HIV in the more advanced stages of disease have been studied (7, 8, 15, 30, 37, 43, 46, 64, 68, 75, 77, 79). In both cases, infected cells are widely distributed in a number of lymphoid and nonlymphoid organs. Though lymphocytes harbor the majority of virus in the peripheral blood (25, 34, 65), cells of the monocyte/macrophage lineage are the predominant site of infection for both HIV and FIV in tissues during advanced-stage disease (7, 8, 30, 43, 46, 64, 75, 77). Determination of very early cellular and tissue targets of immunodeficiency viruses even before the onset of acute-stage illness will be important for furthering our understanding of the pathogenesis of HIV infection.

In the current study, we used in situ hybridization and immunohistochemistry to examine the dissemination and cellular targets of FIV in tissues at various time points throughout the development of the primary acute stage of infection. Key findings were early widespread dissemination of FIV to lymphoid and nonlymphoid organs and a change in the predominant cellular target of FIV in tissues from T lymphocytes to macrophages upon development of the acute clinical syndrome. This early shift to macrophage infection may be important for persistence of viral infection.

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TABLE 1. Clinical, virologic, and serologic status of cats at time of necropsy

Cat no.	Days p.i.[a]	Sex[b]	Age (mo) at inoculation	Status														
				Serology[c]	Virus isolation (PBMC)	Plasma viremia	Neutropenia[d]	Lymphopenia[e]	Enlarged lymph nodes	Fever[f]	Diarrhea	Dehydration	Depression	Soreness	Tonsillitis	Ocular discharge	Icteric discharge	Scruffy coat
5095	5	M	5	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
5109	5	F	5	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
5108	10	F	5	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–
5123	10	M	4	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–
5107	21	F	5	+	+	+	–	–	+	–	–	–	–	–	–	–	–	–
5124	21	F	4	+	+	+	–	–	+	–	–	–	–	–	–	–	–	–
5106	28	F	5	+	+	+	–	–	+	–	–	–	–	–	–	–	–	–
5127	28	M	4	+	+	+	–	–	+	–	–	–	–	–	–	–	–	–
5105	42	M	5	+	+	+	–	–	+	–	+	–	–	–	–	–	–	+
5125	42	F	4	+	+	+	+	–	+	–	+	–	–	–	–	–	–	+
5126	56	F	4	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
5129	56	F	4	+	+	+	+	+	+	+	+	+	+	+	–	+	–	+
5122	70	M	4	+	+	+	+	–	+	+	+	+	+	–	–	–	+	+
5128	70	M	4	+	+	+	+	+	+	+	+	+	+	–	+	–	+	+
5096	84	F	5	+	+	+	–	–	+	–	–	–	–	–	–	–	–	–
5097	84	F	5	+	+	+	–	–	+	–	–	–	–	–	–	–	–	–

[a] Days after inoculation that necropsy was performed on.
[b] M, male; F, female.
[c] +, serum antibodies specific for FIV Gag proteins by ELISA.
[d] Neutropenia, <3,000 neutrophils per μl.
[e] Lymphopenia, <1,500 lymphocytes per μl.
[f] Temperature equal to or above 103°F (ca. 39.4°C).

MATERIALS AND METHODS

Animals and viral infections. FIV infection of the cats used in this study has been described in detail by Dua and coworkers (24). Specific-pathogen-free domestic cats were obtained from the breeding colony of the Feline Retrovirus Research Laboratory, University of California, Davis, and were housed in facilities of the Animal Resource Services. Sixteen cats between the ages of 18 and 24 weeks (Table 1) were inoculated intraperitoneally with 1 ml of whole heparinized blood from cat 5000, a chronic carrier of the Petaluma strain of FIV (Table 1). Cat 5000 appeared healthy but had a low $CD4^+/CD8^+$ T-lymphocyte ratio of 0.33 at the time blood was collected for use as the inoculum in this study. Dua and associates titrated the inoculum by the ability to recover virus by coculture (24). Viral antigen as measured by antigen capture enzyme-linked immunosorbent assay (ELISA) could be detected in cultures diluted up to 1:1,000 with peripheral blood mononuclear cells (PBMC) from uninfected cats (24). Each 1-ml inoculum of blood contained approximately 5×10^7 PBMC, for a dose of about 50,000 infectious cells per cat.

Clinical, hematologic, and serologic evaluation of cats. Cats were evaluated clinically for palpable lymph nodes and clinical signs of illness. Complete blood counts and differentials were performed by standard techniques (2). $CD4^+$ and $CD8^+$ T-lymphocyte counts were determined by flow cytometry as previously described (2). Serum was screened weekly or biweekly for the presence of antibodies to FIV p24 antigen, using an ELISA as described previously (60). Plasma viremia was determined by the ability of plasma to productively infect concanavalin A-stimulated cultures of PBMC from an uninfected cat as described elsewhere (24). Culture supernatants were tested for the presence of FIV p24 by antigen capture ELISA (20). Virus isolation by PBMC culture was performed as previously described (20). Clinical, hematologic, and serologic findings as well as peripheral blood viremia of cats in the present study have been described in detail by Dua and associates (24).

Tissue collection and preparation. Two cats were killed by barbiturate anesthetic overdose, and tissues were collected at each of the following time points: 5, 10, 21, 28, 42, 56, 70, and 84 days after infection (p.i.) (Table 1). Lymph nodes (submandibular, mediastinal, mesenteric, ileocecal, popliteal), spleen, thymus, bone marrow, cerebrum, cerebellum, spinal cord, salivary gland, tonsil, lung, liver, kidney, jejunum, ileum, cecum, and colon were collected. Tissues were fixed in 10% neutral buffered formalin before embedding in paraffin. Sections of 3 to 5 μm were placed on Fisherbrand Superfrost/Plus slides (Fisher Scientific) and stained with hematoxylin and eosin for histopathology.

Detection of FIV nucleic acids by in situ hybridization. The in situ hybridization procedure was performed as previously described for detection of RNA alone (7) or modified to detect RNA and DNA. Briefly, a ^{35}S-labeled probe with a specific activity of greater than 4×10^8 cpm/μg was generated by random priming of the full-length FIV-Petaluma proviral genome (58, 71). Radiolabeled probe was added at 3×10^7 cpm/ml to a hybridization solution containing 50% deionized formamide, 10% dextran sulfate, 50 mM NaH_2PO_4, 0.6 M NaCl, 0.5 mM EDTA, 1× Denhardt's solution, 75 μg of *Escherichia coli* tRNA per ml, 100 μg of salmon sperm DNA per ml, and 20 mM dithiothreitol. Denatured probe in hybridization solution was placed over proteinase K (Sigma)-treated tissue sections (1 μg of proteinase K per ml, 15 min, 40°C), and slides were coverslipped. For detection of viral RNA only,

hybridized slides were incubated overnight at 42°C. For additional detection of viral DNA as well as RNA, coverslipped slides were denatured just prior to overnight incubation by heating at 95°C for 7 min and cooling on ice. After overnight incubation, slides were washed in 50% formamide–2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) followed by 1× SSC at 42°C, dehydrated, and dipped in NTB2 emulsion (Eastman Kodak) diluted 1:2 with 600 mM ammonium acetate. Slides were developed after exposure for 7 to 12 days at 4°C and then counterstained with Mayer's hematoxylin. FIV-infected and uninfected tissues as well as infected and uninfected Crandell feline kidney cells served as controls in each in situ hybridization experiment.

An even background of one to two grains per cell was found on all tissues, contrasting with distinct, well-defined clusters of grains over infected cells. Each tissue section was scored for viral load as follows: + (<4 positive cells per 10× field), ++ (4 to 40 positive cells per 10× field), or +++ (>40 positive cells per 10× field). All tissues were tested a minimum of three times.

Identification of cellular targets by combined in situ hybridization-immunohistochemistry. For simultaneous detection of cell surface proteins and viral nucleic acids, in situ hybridization was followed by immunohistochemistry. In situ hybridization was performed as described above except that (i) slides were fixed for an additional 1 h in 4% paraformaldehyde before protease treatment and (ii) slides were treated with 250 μg of protease XXIV (Sigma) per ml for 15 min at 24°C rather than with proteinase K. After the probe was removed, immunohistochemistry was performed as described below. Slides were then dipped in emulsion, exposed, developed, and counterstained as described for in situ hybridization above. Slides run only through immunohistochemistry or in situ hybridization were included as controls.

Immunohistochemistry was performed at room temperature, using a labeled streptavidin biotin kit (Dako Corp.). Tissues were blocked with normal goat serum and then incubated with one of the following primary antibodies for 1 h. A polyclonal rabbit anti-human CD3 antibody (Dako Corp.) diluted 1:50 to 10 μg/ml was used to detect T lymphocytes. The peptide that this antibody was made to, a 13-mer from the intracytoplasmic domain of the CD3ε (51), is highly conserved, showing 100% homology between humans and mice (40). Antibodies to this peptide detect T lymphocytes in tissues from a variety of species (40). The morphology and distribution of stained cells in feline tissues were consistent with those of T lymphocytes. Results were validated with polyclonal rabbit anti-bovine papillomavirus antibody (B580; Dako Corp.) at a 20-μg/ml total immunoglobulin concentration as a nonspecific primary antibody control. Monoclonal antibody Mac387 (Dako Corp.) diluted 1:100 to 0.85 μg/ml was used to detect monocytes, macrophages, and granulocytes. Mac387 detects a myeloid antigen which has been partially characterized in human tissues. Mac387 is a useful marker for monocyte/macrophage differentiation in formalin-fixed, paraffin-embedded tissue sections. The morphology and distribution of stained cells in feline tissues were consistent with those of monocytes, macrophages, and granulocytes. Granulocytes are readily distinguished from monocytes/macrophages by nuclear morphology. A pool of ascites fluid from three mouse monoclonal antibodies (24-IA_2, 24-IB_3, and 24-IIG_3) recognizing p27 of feline leukemia virus was used at a concentration of 225 μg/ml on duplicate slides as a nonspecific isotype-matched control (48). Following incubation with primary antibody, slides were incubated for 10 min with a mixture of biotinylated goat anti-mouse and biotinylated goat anti-rabbit immunoglobulin, quenched for 30 min in 0.45% H_2O_2 in 0.1% sodium azide–Tris-buffered saline, and then incubated for 10 min with streptavidin conjugated to horseradish peroxidase. Immobilized antibody/streptavidin-horseradish peroxidase complexes were detected with 3′-amino-9-ethylcarbazole (Vector Laboratories) as a chromagen. Tissues were counterstained with hematoxylin to visualize nuclei.

Percentages of FIV in situ hybridization-positive cells that stained as either monocytes/macrophages or T lymphocytes were estimated from a minimum of two experimental runs per tissue.

RESULTS

Clinical, hematologic, peripheral virologic, and serologic status of cats. Clinical, hematologic, and serologic findings as well as peripheral blood viremia of cats are summarized in Table 1 and have been described in detail by Dua and associates (24). Enlarged lymph nodes were the first noticeable sign after FIV infection, beginning on day 10 and continuing throughout the study period of 12 weeks. Popliteal lymph node size peaked around days 42 to 56 at up to 1-cm diameter. Flu-like illness characterized by fever, diarrhea, dehydration, and depression began on day 42 and was most severe 56 days p.i. Leukopenia associated with lymphopenia and neutropenia was first evident at 42 days p.i. and was most severe on day 56 (24). $CD4^+/CD8^+$ T-lymphocyte ratios began decreasing by day 42 p.i. as well (24). PBMC-associated virus and plasma virus was detectable beginning days 14 and 21 p.i., respectively, and continuing throughout the 84-day study period (24). Serum antibodies to FIV were first detected by ELISA 14 days p.i. (24).

Histopathology. The most striking and consistent histopathologic lesions were observed in peripheral lymphoid organs, which included peripheral lymph nodes, spleen, and lymphoid tissues associated with mucosal surfaces (tonsil, mesenteric lymph nodes, aggregated lymphoid follicles of ileum and cecum, and solitary lymphoid follicles of duodenum, jejunum, and colon) (Table 2). These lymphoid organs showed progressive hyperplastic changes consisting of increased number and/or size of lymphoid follicles with prominent germinal center formation. Germinal centers were occasionally misshapen (Fig. 1A), and mantle lymphocytes were frequently depleted. Parafollicular domains, which included the paracortical zones of lymph nodes and the periarterial lymphoid sheath of the spleen, were also expanded by lymphocytes, lymphoblasts, and macrophages. Paracortical high endothelial venules were usually prominent and contained numerous lymphocytes within the vessel wall (Fig. 1B). Sinus hyperplasia and medullary cord hyperplasia were also prominent. Plasmacytosis of medullary cords with variable extension to the paracortex were also frequently observed. Expansion of the marginal zone at the periphery of the splenic white pulp by lymphocytes and macrophages was commonly observed, and myeloid metaplasia of the splenic red pulp was a consistent and often striking change. The magnitude of these lesions was greater with time, and the most florid lesions were seen beyond 42 days p.i., concomitant with the onset of clinical illness.

Histopathologic lesions were also encountered in central lymphoid organs. Marked thymic cortical involution, characterized by almost complete disappearance of cortical thymocytes, was increasingly evident beyond 42 days p.i. This cortical involution coincided with the decline in peripheral blood CD4 count (24). Thymitis, manifest by interlobular infiltration by lymphocytes and macrophages which encroached on the adjacent thymic cortex, was also present (Fig. 1C and D). Lym-

TABLE 2. Histopathologic lesions in cats in the early stage of FIV infection

Cat no.	Days p.i.	Histopathologic lesions[a]															
		Bone marrow	Thymus			Peripheral LN	Tonsil	Spleen		Mesenteric LN and MALT	Large intestine	Kidney	Lung	Liver	Cerebrum	Spinal cord	
		Myeloid hyperplasia	Cortical involution	Thymitis	Follicular hyperplasia	Lymphoid hyperplasia	Lymphoid hyperplasia	Lymphoid hyperplasia	Myeloid metaplasia	Lymphoid hyperplasia	Typhlitis	Interstitial nephritis	Interstitial pneumonia	Periportal hepatitis	Encephalitis	Myelitis	
5095	5	−	−	−	−	+	+	+	+	+	+	−	−	−	−	−	
5109	5	−	−	−	−	+	0	−	+	+	+	−	+	−	−	−	
5108	10	−	−	−	−	+	+	−	+	+	−	−	+	−	−	−	
5123	10	−	−	−	−	+	+	−	+	+	0	−	−	−	−	−	
5107	21	−	−	−	−	+	+	+	++	+	+	−	+	−	−	−	
5124	21	−	−	−	−	+	−	+	+	+	−	−	+	+	−	−	
5106	28	−	−	−	−	+	+	+	+	+	+	−	+	−	−	−	
5127	28	−	−	−	−	+	−	+	+	+	+	+	−	−	−	−	
5105	42	−	+	+	−	+++	++	+++	+	+++	−	++	++	−	−	−	
5125	42	−	+	+	−	++	++	++	+	+++	−	−	++	−	−	−	
5126	56	+	+++	+	−	++	++	++	++	−	+++	+	+	−	+	−	
5129	56	+	+++	+	−	++	+	++	++	++	++++	+	−	−	−	−	
5122	70	+	+++	+	+	++	++	++	+	++	++	++	+	+	−	−	
5128	70	++	+++	+	+	+++	++	++	+++	++	++	+	++	+	−	+	
5096	84	−	+	−	+	++	0	++	+	+++	+	+	+	+	−	−	
5097	84	−	+++	+	+	++	++	++	+	+++	+	−	−	−	−	−	

[a] LN, lymph node; MALT, mucosa-associated lymphoid tissue; 0, tissue not present.

phoid follicular hyperplasia of the thymic medulla was consistently observed in the four cats sampled after 70 days p.i. These follicles also possessed germinal centers. Lymphoid follicular hyperplasia of the bone marrow was also observed late in the study. The bone marrow was also hyperplastic in cats sampled at days 56 and 70. This was manifest as an increased cellularity of the marrow and an increase in the proportion of granulocyte and monocyte precursors. A mature granulocyte reserve was not demonstrable in cat 5128 (day 70).

Histopathologic lesions were also encountered in nonlymphoid organs. Perhaps the most striking of these lesions was the transmural typhlitis that consistently developed in cats beyond 56 days p.i. In its most florid form, this lesion consisted of a marked pleocellular inflammatory cell infiltrate which was especially severe in the submucosa of the cecum adjacent to and within the lymphoid follicles (Fig. 1E). These lesions often obliterated the submucosal lymphoid follicles or resulted in marked lymphoid depletion. Large vesicular mononuclear cells distributed around vessels were particularly prominent. Invasion of vessel walls by these mononuclear cells was evident in cat 5126 (day 56). The inflammatory infiltrate, which also contained neutrophils, lymphocytes, plasma cells, and eosinophils in variable proportions, extended through the muscular coats to the serosa (Fig. 1F and G). Venous thrombosis as well as necrosis of infiltrating cells and the involved tissues of the submucosa and muscularis were observed in severe lesions. Inflammatory infiltrates were not as prominent in the lamina propria, but when present and severe, they were accompanied by mucosal erosion and ulceration.

Inflammatory disease of other organs also occurred. Pulmonary interstitial inflammatory infiltrates and renal cortical interstitial infiltrates were quite commonly observed. Brain lesions were seen only in cat 5126 (day 56). This animal had prominent vascular lesions characterized by lymphocytes and large mononuclear cells infiltrating vessel walls in the cerebral cortex (Fig. 1H). Similar vascular lesions were observed in the tonsil and in the cecum in this animal.

Dissemination of FIV in tissues. FIV nucleic acids were first detected by in situ hybridization in tissues of healthy cats necropsied 21 days p.i. (Table 3), correlating with the first appearance of infectious virus in the plasma (24). Other than enlarged lymph nodes, cats did not have other signs of acute illness until day 42 p.i., 3 weeks after virus was detected in plasma, PBMC, and tissues. The vast majority of FIV-infected cells were present in lymphoid organs, while low numbers of infected cells, generally less than 15 per organ cross section, were detected in some nonlymphoid organs. The two cats with the most severe illness (cats 5126 and 5129) had a slightly higher viral load in nonlymphoid organs, though lymphoid organs in these cats remained the predominant reservoirs of FIV-infected cells.

Lymphoid tissues, including lymph nodes, spleen, gut-associated lymphoid tissue, bone marrow, thymus, and tonsils, harbored the majority of FIV-infected cells in cats before and during the acute primary illness caused by FIV infection. FIV-infected mononuclear cells were often located in germinal centers of lymphoid follicles in lymph nodes, spleen, and gut-associated lymphoid tissue. In many lymph nodes, more than 50% of infected cells were located in germinal centers (Fig. 2A). Infected cells were also often present in the paracortex and medullary cords of lymph nodes and less often in sinuses. In the spleen, infected cells were present in periarterial lymphoid sheaths and in the red pulp as well as in activated germinal centers (Fig. 2B). FIV-infected cells were present in Peyer's patches and lamina propria and at high levels in inflammatory lesions in the intestine (Fig. 2C). Both mononuclear cells and megakaryocytes, as identified by size and characteristic nuclear morphology, were infected in the bone marrow (Fig. 2D). Infection was often heavy in the thymus,

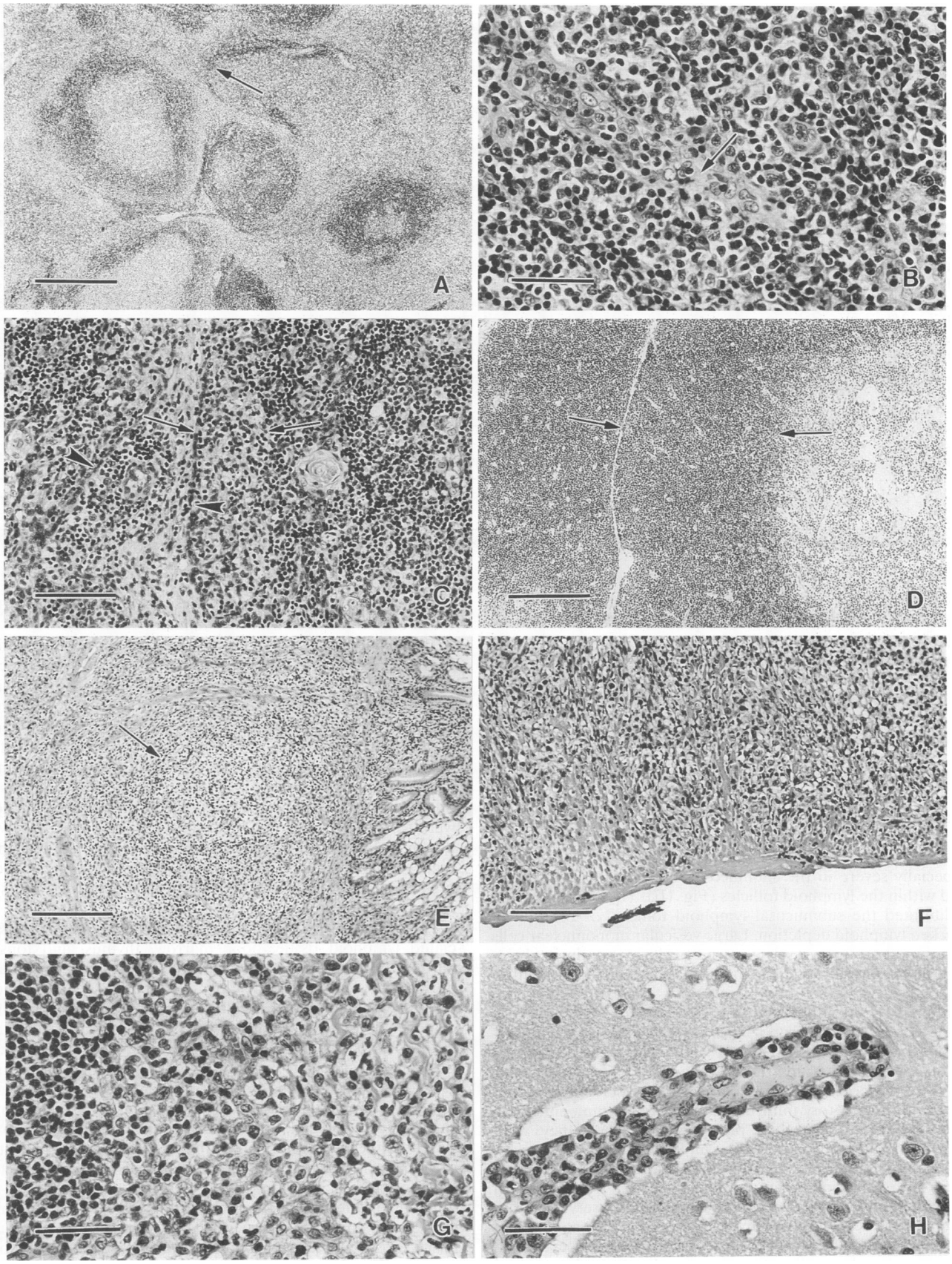
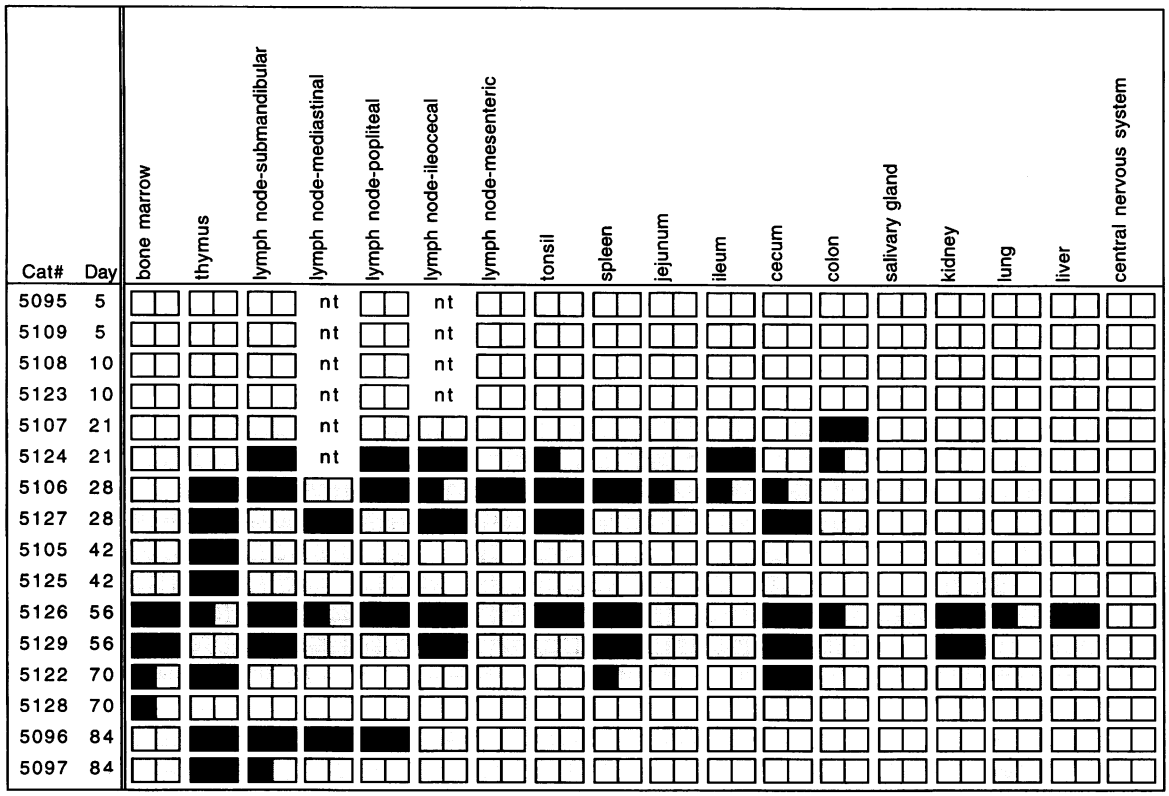

A
B
C
D
E
F
G
H

TABLE 3. Presence of FIV nucleic acids in tissues as determined by in situ hybridization[a]



[a] Left-hand box and right-hand box for each tissue tested, RNA-DNA and RNA only, respectively. □, no positive cells; ▩, <4 positive cells per 10× field; ■, 4 to 40 positive cells per 10× field; ■, >40 positive cells per 10× field; nt, not tested.

with the majority of infected cells located in the thymic cortex (Fig. 2E).

Infected cells in nonlymphoid organs were rare and appeared to be either interstitial mononuclear cells or mononuclear inflammatory cells (Fig. 2F to H). The majority of FIV-infected cells in nonlymphoid organs were present in the lung (Fig. 2F), liver, and kidney (Fig. 2G). These infected cells were present in the interstitium, often around ducts or infiltrating blood vessels or in areas of inflammation. One cat (cat 5126) had FIV-infected cells in the choroid plexus of the brain and associated with vascular lesions in the cerebral cortex (Fig. 2H).

The majority of FIV-infected cells in the tissues of cats during the first 12 weeks after FIV inoculation were determined to be productively infected because the numbers of positive cells detected by RNA in situ hybridization were similar to those detected by RNA-DNA in situ hybridization. Approximately 15% of tissues tested had fewer positive cells by RNA in situ hybridization than by RNA-DNA in situ hybridization, indicating either latent infection or low-level replication in many infected cells. No clear pattern of latent or weakly productive infection could be discerned.

Cellular targets of FIV. Early after infection, before the onset of clinical signs other than enlarged lymph nodes, 25 to 75% of the infected cells in most tissues were T lymphocytes (Fig. 3A to D). Rare infected cells of the monocyte/macrophage lineage were also detected. Beginning with the peak of flu-like illness on day 56, a dramatic increase in the proportion of infected cells identified as macrophages was observed (Fig. 3E and F). While most tissues still contained a small popula-

FIG. 1. Histopathologic examination of lesions in cats early after FIV infection. (A) Popliteal lymph node. Marked lymphoid follicular hyperplasia; the uppermost lymphoid follicle shows some lymphocyte depletion (arrow) in the follicular mantle. (Bar, 500 μm; cat 5128, 70 days p.i.) (B) Popliteal lymph node. Paracortical lymphoid hyperplasia; note the prominent high endothelial venule in the center of the field (arrow). The paracortical region was expanded by lymphocytes and histiocytes. Plasma cells were also observed in low numbers. (Bar, 50 μm; cat 5128, 70 days p.i.) (C) Thymitis and thymic cortical atrophy. The interlobular septum (bounded by arrowheads) is infiltrated by lymphocytes and histiocytes; the cortex (bounded by arrows) is markedly attenuated and almost devoid of thymocytes. (Bar, 100 μm; cat 5126, 56 days p.i.) (D) Thymus from a cat earlier after infection. This thymus is relatively normal in appearance; note the lack of infiltrate in the interlobular septum and the prominent cortex (bounded by arrows) which is replete with thymocytes. (Bar, 200 μm; cat 5106, 28 days p.i.) (E) Cecum with transmural typhlitis. Submucosal lymphoid nodule with lymphoid depletion (arrow) and surrounding pleocellular inflammation. (Bar, 200 μm; cat 5129, 56 days p.i.) (F) Cecum with transmural typhlitis. Muscularis and serosa with transmural pleocellular inflammatory infiltrate. (Bar, 100 μm; cat 5129, 56 days p.i.) (G) Cecum with transmural typhlitis. Inflammatory infiltrate adjacent to a submucosal lymphoid follicle. The infiltrate consists of histiocytes, lymphocytes, and a few granulocytes. (Bar, 50 μm; cat 5126, 56 days p.i.) (H) Cerebral blood vessel with a lymphohistiocytic infiltrate in the vessel wall; some of the infiltrating cells are shrunken and pyknotic. (Bar, 50 μm; cat 5126, 56 days p.i.)

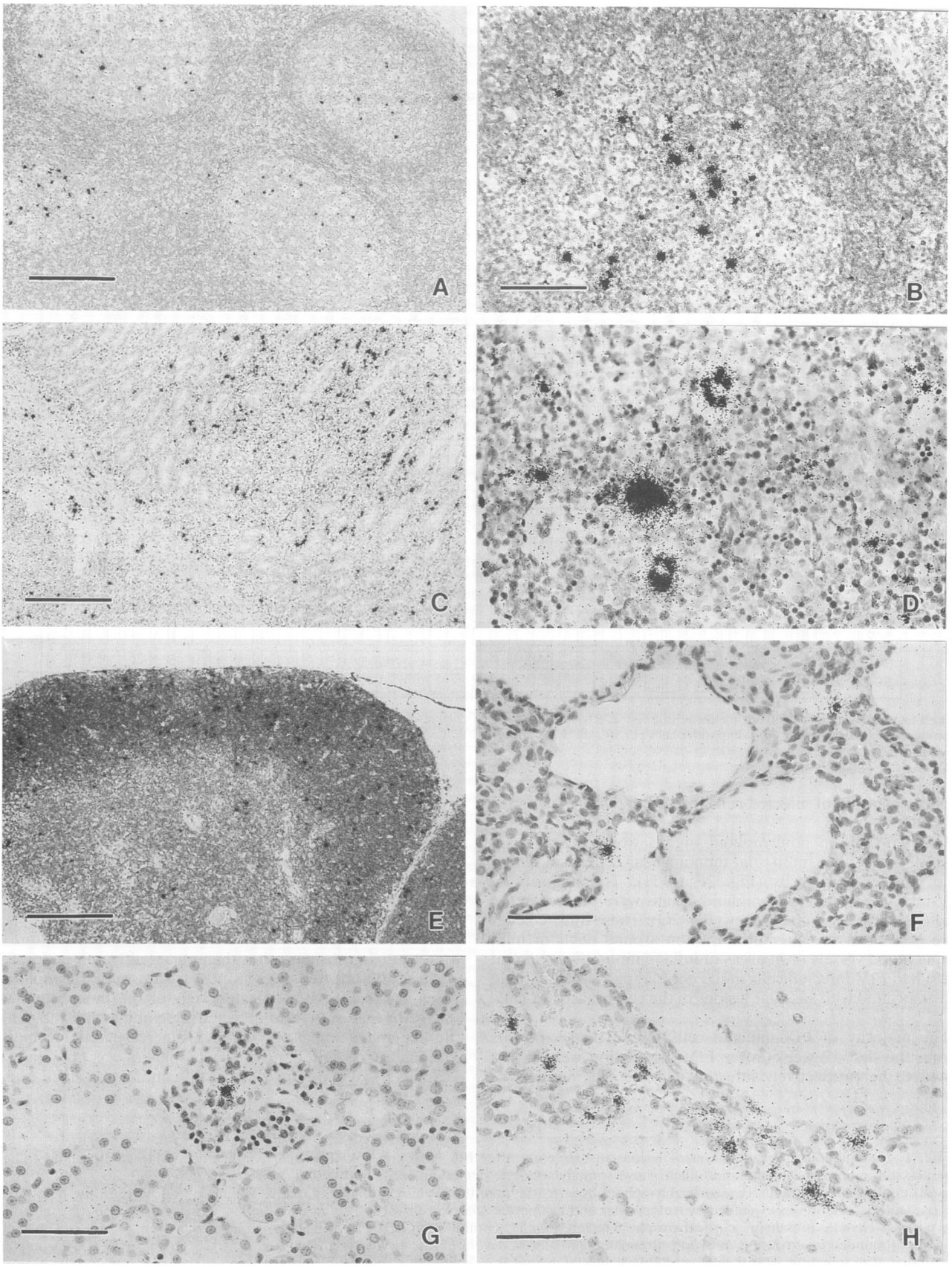


FIG. 2. Localization of FIV nucleic acids in tissues from FIV-infected cats as detected by in situ hybridization. FIV nucleic acids are detected by silver grains overlying the cells. FIV infection is shown in cells within the germinal centers of the submandibular lymph node from cat 5096 (A; bar, 240 μm), within a germinal center of the spleen in cat 5106 (B; bar, 100 μm), associated with typhlitis in the lamina propria and submucosa of the cecum of cat 5126 (C; bar, 240 μm), in the bone marrow of cat 5128 (D; bar, 60 μm), in the thymic cortex of cat 5127 (E; bar, 240 μm), in the lung of cat 5125 (F; bar, 60 μm), in a glomerulus in the kidney of cat 5127 (G; bar, 60 μm), and in infiltrating cells within the wall of a blood vessel in the brain of cat 5126 (H; bar, 60 μm).

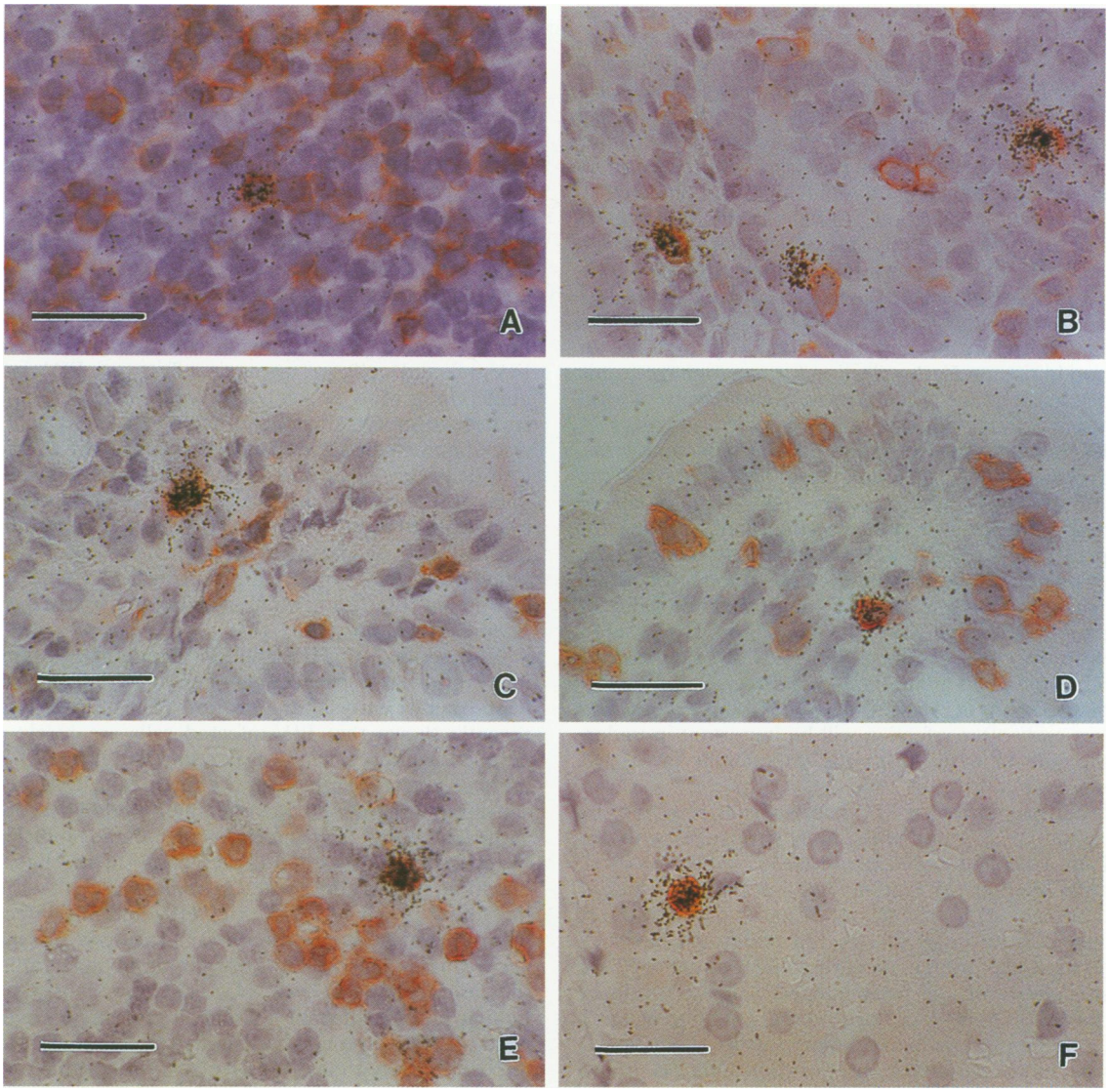


FIG. 3. Cellular targets of FIV in tissues of cats early after FIV infection. FIV nucleic acids were detected by in situ hybridization with a radiolabeled probe and are indicated by silver grains overlying the cells. Immunohistochemistry was used to label T cells or monocytes/macrophages (red stain). Early after infection, cats had many FIV-infected T cells, as demonstrated in the lymph node of cat 5125 (A; bar, 30 μm), the ileum of cat 5124 (B; bar, 30 μm), the colon of cat 5124 (C; bar, 30 μm), and the jejunum of cat 5124 (D; bar, 30 μm). Once cats became clinically ill, the majority of FIV-infected cells were of the monocyte/macrophage lineage, as demonstrated in the lymph node of cat 5129 (E; bar, 30 μm) and the liver of cat 5096 (F; bar, 30 μm).

tion of FIV-infected T lymphocytes, normally 2 to 10% of infected cells, the majority of the FIV-infected cells, 40 to 95% depending on the tissue, were of the monocyte/macrophage lineage. A significant proportion of the infected cells in some tissues were mononuclear cells that could not be identified as T lymphocytes or macrophages with the antibodies used.

DISCUSSION

The objective of this study was to identify early cellular and organ targets of FIV infection in vivo both preceding and during the acute primary illness. The appearance of FIV-infected cells in tissues came 1.5 weeks after the enlargement of lymph nodes and 3 weeks before the occurrence of flu-like clinical signs. Primary and secondary lymphoid organs, including thymus, bone marrow, lymph nodes, spleen, tonsil, and lymphoid tissues of the intestinal tract, harbored the majority of FIV-infected cells before and during the primary stage of infection; low levels of virus were present in nonlymphoid organs, including lung, liver, kidney, brain, spinal cord, and pancreas. Thus, the viral infection was already widely disseminated and viral load had reached high levels in lymphoid tissues weeks before clinical signs of illness appeared.

Early cellular targets of FIV in tissues of cats before clinical signs of primary illness included many T lymphocytes (25 to 75% of infected cells), a few macrophages (2 to 10% of infected cells), and an unidentified population of mononuclear cells; however, the proportion of infected macrophages increased dramatically to 40 to 95% of the infected cells during the acute flu-like illness, replacing T lymphocytes as the primary site of viral infection. We have recently demonstrated

that the majority of infected cells in tissues of terminal-stage cats are also cells of the monocyte/macrophage lineage (8). Therefore, although T lymphocytes appear to be an important early target in tissues, the high viral load that accompanies either primary or terminal clinical illness is associated largely with macrophages. The observed shift in predominant cellular targets from T lymphocytes to macrophages with the onset of primary disease may be due to rapid killing of infected T lymphocytes leading to selection of viral variants that preferentially infect or replicate in cells of the monocyte/macrophage lineage. Like HIV, FIV is known to be highly cytopathic to T lymphocytes in vitro (4, 12, 56), while macrophages are less susceptible to the cytopathic effects (13, 64). Macrophage infection may therefore be important for the persistence of FIV infection in tissues. Early selection for macrophage-tropic variants may account for the observed homogeneity of viral variants during primary disease in HIV-infected patients (78, 80, 81) despite extensive variation in the infecting virus. Studies of primary HIV infection have suggested that the earliest viral variants present in the peripheral blood of HIV-infected patients are non-syncytium-inducing macrophage-tropic variants (63, 80, 81). However, the earliest time points investigated in these studies were during the acute primary illness. The present study demonstrates the value of examining viral genotypic and phenotypic properties even earlier, before the onset of clinical illness, which can be done systematically only in an animal model. Biological and molecular characterization of viruses derived from T cells and macrophages early after infection will be important in understanding the role of early host-virus interactions in disease pathogenesis.

A significant proportion of FIV-infected cells could not be identified as either T lymphocytes or macrophages with the polyclonal anti-CD3 and monoclonal Mac387 antibodies used, indicating that another cell type may be infected in tissues of cats in the primary stage of infection. English and coworkers have recently demonstrated that in the peripheral blood, T lymphocytes are the predominant early cellular target of FIV 2 to 4 weeks p.i.; however, this predominance shifts to B cells by 11 weeks p.i. (25). This work corroborates our finding of T lymphocytes as the predominant cellular targets in tissues early after infection but not after the onset of clinical illness. It also raises the question of whether B cells may be infected in the tissues. It is possible that many of the unidentified FIV-infected cells within tissues may be B cells or plasma cells. Another possibility is that the heterogeneity of cells of the monocyte/macrophage system results in the inability to detect all cells of this lineage with one antibody. Monoclonal antibody Mac387 is known to detect many, but not all, of the various members of the monocyte/macrophage family. For example, Mac387 does not appear to stain dendritic cells in paracortical zones of lymph nodes, in the periarterial lymphoid sheath of spleens, or in skin. The interstitial location of many of the unidentified FIV-infected cells suggests that they may be interdigitating reticulum cells in lymphoid organs or interstitial dendritic cells in nonlymphoid organs.

Primary lymphoid organs were a major site of FIV infection in the early stage of disease. FIV infection of bone marrow was seen as early as 21 days p.i. Consistent with earlier reports, the level of FIV infection in bone marrow was highest in cats with the most severe clinical signs and hyperplastic marrow (days 56 and 70) (7, 8). Similar findings of hyperplasia and heavy infection of the bone marrow have been observed early after SIV infection (49, 50) and in later stages of HIV infection (82, 83). FIV was detected in the thymuses of all cats necropsied day 21 and beyond. Viral load in the thymus was high early in many cats and was localized almost exclusively to the cortex. Cortical involution was observed later, beginning day 42, and increased in severity with time, correlating with depletion of $CD4^+$ T lymphocytes in the peripheral blood (24). Follicular hyperplasia was noted in thymuses of cats necropsied days 70 and 84. Cortical involution and follicular hyperplasia have been reported in HIV-infected adults, children, and fetuses (32, 41, 55, 59) and in SIV-infected macaques (5, 52), though high viral load has not been demonstrated. FIV-infected cats had a high viral load in the thymic cortex early after infection followed by cortical involution. The decrease in viral load upon onset of cortical involution appears to be due to the absence of viral targets in the involuted cortex. Cortical involution following high viral load in the thymic cortex may be due to death of FIV-infected cells or cytotoxic effects of FIV infection on neighboring cells. A similar extensive infection and death of cortical thymocytes has been demonstrated upon HIV infection of human fetal thymus-liver explants in SCID mice (1, 10). Cell death in the thymus-liver explants was associated with a high viral load. Immature CD4 CD8 double-positive thymocytes were found to be the major target of HIV infection and cytopathicity in the thymuses of these mice. Thus, early infection of the thymus may contribute to $CD4^+$ lymphocyte attrition by interfering with the ability of the host to regenerate mature T lymphocytes.

As described for HIV and SIV infections, germinal centers harbored a large proportion of the FIV-infected cells in the lymph nodes early after infection (27, 44, 54, 62). We have recently shown that FIV-infected cells are rarely present in germinal centers in the lymph nodes of terminally ill cats but are concentrated instead in the medullary, intermediate, and subcapsular sinuses, paracortex, and medullary cords (8). As with HIV and SIV infections, lymphoid follicles become hyperplastic early after FIV infection; in the later stages of infection, lymph nodes often become depleted, with follicular involution (9, 11, 14, 23, 47, 61). HIV and SIV infections have been demonstrated in the germinal centers of lymph nodes early, when lymph nodes are hyperplastic, but not later, when they are depleted (27, 44, 54, 62). This is consistent with our finding of FIV-infected cells in the germinal centers of the hyperplastic lymph nodes examined in this study. However, in our FIV-infected cats, the virus within germinal centers appeared to be associated only with mononuclear cells, whereas in HIV and SIV infections, much of the virus appears as free virus associated with follicular dendritic cells (FDC) (27, 39, 46, 54, 62, 73). FIV infection of FDC in the lymph nodes of two of three asymptomatic cats 5 months after experimental inoculation has recently been demonstrated by immunohistochemical staining (74). Differences between these and our results may be due to the different techniques used to detect virus, differences in viral inoculum or route of inoculation, or the stage of infection examined. In any case, the FDC network may play an important role in sequestering either infected cells or free virus within the germinal centers. Eventually, disruption of the FDC network may result in involution of follicles and release of sequestered virus.

In nonlymphoid tissues, infected cells were commonly present in the interstitium especially around ducts, in blood vessels and associated with vascular lesions, or in inflammatory lesions. Cells other than interstitial mononuclear leukocytes or mononuclear inflammatory cells did not appear to be infected. The association of high viral load with inflammatory or vascular lesions was particularly evident in transmural typhlitis and in vascular lesions in the brain. Similar vascular lesions have been observed in the brains of macaques within weeks of SIV infection (66) and in the brain of a patient 15 days after

accidental HIV infection (21). SIV- or HIV-infected cells were often present in these vascular lesions. Intestinal abnormalities accompanied by infection of cells within the lamina propria and submucosa are also an early complication of SIV infection (35, 36). FIV-infected interstitial cells were often, but not always, associated with inflammatory lesions in kidney (interstitial nephritis), lung (interstitial pneumonia), and liver (periportal hepatitis). A prospective study of the early events in SIV infection demonstrated a similar periportal hepatitis and interstitial pneumonia accompanied by SIV infection (6, 31). Thus, the early pathologic and virologic findings in FIV infection are in many ways similar to those in SIV and HIV infection.

Our finding of FIV-infected cells in nonlymphoid organs very early after infection indicates that a widespread dissemination of virus occurs early during the acute primary illness following infection. We have recently shown heavy infection in nonlymphoid organs of terminally ill experimentally FIV-infected cats (8). However, while viral burden is often high in lymphoid tissues even before the cats become clinically ill, viral load in nonlymphoid organs appears to remain relatively low. Studies in HIV-infected individuals suggest that distinct viral strains with different molecular and biologic characteristics can evolve independently within different tissues of an infected host (3, 16, 26, 33, 69, 72). Therefore, the early seeding of virus to many different tissues may lead to the development of viral variants that play an important role in disease pathogenesis.

Evidence is provided in this report for (i) early dissemination of FIV to lymphoid and nonlymphoid organs and (ii) a change in the predominant cellular targets of FIV in tissues from T lymphocytes to macrophages upon development of primary clinical illness. This study demonstrates the importance of studying the events prior to the onset of primary stage illness. FIV-infected cats should provide an excellent model for studying early host-virus interactions, including initial immune response to viral infection and selection for viral variants in a range of different tissues beginning very early after infection. Such studies may provide valuable contributions to our understanding of the pathogenic mechanisms of immunodeficiency-inducing lentiviruses.

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