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Feline Immunodeficiency Virus Predisposes Cats to Acute Generalized Toxoplasmosis

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This study was designed to examine the effects of a pre-existing, clinically asymptomatic feline immunodeficiency virus (FIV) infection on a primary challenge with* Toxoplasma gondii. *Parenteral challenge of FIV-infected cats with tachyzoites of the ME49 strain of* T. gondii *caused a precipitous drop in all lymphocytes ($CD4^+$, $CD8^+$, and B cells) and generalized severe toxoplasmosis. The predominant postmortem lesions included acute and often fatal interstitial pneumonia, dominated histologically by macrophages, and multifocal to coalescing hepatic necrosis. Immunohistochemistry revealed numerous* T. gondii *antigen and tachyzoites in macrophages and other cell types in the lung lesions. The proliferative response of peripheral blood mononuclear cells to specific (*T. gondii *antigen) and nonspecific (Concanavalin A) mitogens was defective in the dually infected cats, suggesting marked immunosuppression. In contrast to the dually infected cats, cats infected only with* T. gondii *developed a transient, mild clinical disease characterized by anorexia, lethargy, and multifocal chorioretinitis. Lymphocyte changes in* T. gondii*-infected cats included an early panlymphopenia followed by reestablishment of all lymphocyte subset profiles. These cats also showed a reduced proliferative response to Concavalin A at 1 week after challenge, but a measurable* in vivo *response to* T. gondii *antigens, as evidenced by* in vitro *lymphocyte proliferation in the absence of a mitogenic stimulus. These results show that infection of cats with FIV-NCSU$_1$ markedly enhances their susceptibility to a primary* T. gondii *infection and provides a model to study the mechanisms of the underlying immunological defect(s) occurring early after HIV infection that may predispose individuals to development of acquired immunodeficiency syndrome and associated diseases. (Am J Pathol 1993, 143:1486–1497)

Infection of individuals with human immunodeficiency virus (HIV) produces multiple effects on the immune system that ultimately lead to dysfunction of humoral and cell-mediated immunity.[1] Phenotypically, HIV infections are characterized by a gradual depletion of $CD4^+$ lymphocytes and, in some cases, an increase in $CD8^+$ cells, the latter of which is thought to be a suppressor T cell response to HIV antigens.[2,3] The progressive decline in $CD4^+$ cells is associated with the development of opportunistic infections, usually of the type that are controlled by $CD4^+$-dependent, cell-mediated immune responses, including activation of macrophages.[4] One such infection is by the protozoan parasite *Toxoplasma gondii*. A large portion of the human population is infected with *T. gondii*, but, because of an effective cellular immune response to the parasite, most of these infections are asymptomatic.[5] However, if the cellular immune system is compromised, as is seen late in HIV infection, for example, latent *T. gondii* infection can be reactivated and clinical toxoplasmosis can develop.[6] While a direct correlation exists between progressive decline in $CD4^+$ lymphocyte numbers and development of clinically evident opportunistic infections,[4] the precise defect(s) in the $CD4^+$ cells and at what time after HIV infection the defect(s) occur have not been established.

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While it is evident, at least from HIV studies, that latent infections such as *T. gondii* and *Mycobacterium* can be reactivated after long-term HIV infection, it is not clear whether HIV induces more subtle immunological defects early after infection that may predispose individuals to severe primary infections with these agents. Critical to a better understanding of the pathophysiology of HIV-induced immune dysfunction is an animal model in which the various components of the cell-mediated immune system can be phenotypically and functionally evaluated in response to both the immunodeficiency-inducing virus and the potential opportunistic agent. Existing data suggest that feline immunodeficiency virus (FIV) infection in cats may provide such a model.

FIV is a lentivirus with biochemical and molecular characteristics similar to HIV, and, like HIV, it infects $CD4^+$ lymphocytes and macrophages. The pathogenesis and clinical disease pattern associated with FIV parallel that seen in HIV infection. Cats develop an acute infection syndrome similar to that seen in HIV-1,[1] including low-grade fever and transient generalized lymphadenopathy,[7] followed by a long asymptomatic period in which the $CD4^+$:$CD8^+$ ratio declines due to a progressive decrease in $CD4^+$ cells.[8–10] In naturally infected cats, this asymptomatic period is followed by the development of a variety of disorders associated with immunodeficiency, including chronic gingivitis/stomatitis, chronic upper respiratory infections, chronic enteritis, and recurrent ocular disease.[11–14]

Also similar to humans, infection of the immunocompetent cat with *T. gondii* typically leads to a latent infection, whereas disseminated toxoplasmosis can occur in immunosuppressed cats.[15] Indeed, a substantial number of cats with presumed clinical toxoplasmosis are also infected with FIV.[16,17] It is not known whether these are primary *T. gondii* infections or reactivated infections due to a compromised immune system.

Because *T. gondii* is an important opportunistic pathogen in both humans with HIV infection and cats with FIV infection, we used the FIV infection-*T. gondii* challenge model to test the hypothesis that immune dysfunction occurs relatively early after FIV infection that may predispose cats to clinical AIDS-like disease after exposure to a relatively avirulent infectious agent. We report herein that as early as 18 weeks after infection with FIV, sufficient impairment of the immune system occurs to convert a normally avirulent primary *T. gondii* infection into acute toxoplasmosis in which the primary life- threatening pathologic lesion is interstitial pneumonia.

Materials and Methods

Cats

Specific pathogen free (SPF) cats were obtained from Liberty Laboratories (Liberty Corner, NJ). All cats were seronegative by enzyme-linked immunosorbent assay (ELISA) for feline leukemia virus (IDEXX, Portland, ME), FIV (IDEXX), and *T. gondii*[18] at the beginning of the study.

Cell Cultures

An interleukin-2 (IL-2)-dependent, CD4-positive lymphocyte culture (FCD4-D) has been established in our laboratory through long-term culture of peripheral blood mononuclear cells (PBMC) from an SPF cat in the presence of recombinant human IL-2 (kindly provided by Hoffman LaRoche, Nutley, NJ). These cells are 100% positive for the feline pan-T cell marker 1.572,[19] 60 to 65% positive for the feline CD4 homologue as recognized by antibody CAT30A,[19] and negative for the CD8 homologue recognized by antibody 3.357.[19] These cells are highly permissive for FIV infection and were used to grow the FIV inoculum. Cells were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum and 40 U/ml of recombinant human IL-2.

Infection with FIV

The FIV-$NCSU_1$ was originally isolated from a naturally infected cat at the North Carolina State University College of Veterinary Medicine and has been described in detail elsewhere.[9,20] PBMC from the FIV-$NCSU_1$ source cat were inoculated into two adult SPF cats to provide a larger pool of cells for inoculum. Co-cultures of PBMC from all three cats with PBMC from SPF cats yielded Mg^{2+}-dependent but not Mn^{2+}-dependent reverse transcriptase (RT) activity, indicating the cells were infected with FIV but not with feline leukemia virus or syncytia-forming virus. Virus inoculum for this study was obtained by co-culturing 1×10^6 pooled PBMC from the three cats described above with 2×10^6 FCD4-D cells in the presence of 100 U recombinant human IL-2 (Hoffman LaRoche) for 10 days. Cell-free viral supernatant with high FIV RT activity was harvested between 6 and 10 days by centrifugation, filtered through a 2-μ filter, and stored at −135 C. An aliquot of pooled supernatant was assayed for infectious virus by the 50% tissue culture infectious dose ($TCID_{50}$) method on feline FCD4-D lymphocyte cultures. The viral supernatant had a $TCID_{50}$ of

6×10^6/ml. Eight 12-week-old SPF cats were inoculated intravenously with 100 µl (6×10^5 $TCID_{50}$) of the viral supernatant 18 weeks before infection with *T. gondii*.

Infection with T. gondii

This feline model was developed by Davidson et al.[21] to study the pathogenesis of primary *T. gondii*-induced ophthalmic disease and used herein to study the effect of a pre-existing FIV infection on this model. Oocysts of the ME49 strain of *T. gondii* stored in 2% sulfuric acid (originally supplied by Dr. J. P. Dubey, USDA, Beltsville, MD) were used to generate organisms for inoculation. Tachyzoites were harvested through described, standard serial mouse inoculation procedures.[22] Briefly, oocysts were washed with Hanks' balanced salt solution three times to remove the sulfuric acid and 100 µl (approximately 50,000 oocysts) were inoculated intraperitoneally into retired breeder white Swiss mice (Charles River Laboratories). Triamcinolone (0.125 mg) was given subcutaneously in the mice to encourage tachyzoite replication. Peritoneal fluid was collected aseptically and a sample examined after microcentrifugation to screen for bacterial contamination. Tachyzoites were enumerated on a hemacytometer and the peritoneal fluid was diluted to 10,000 organisms/ml. Peritoneal harvests were inoculated into cats within 45 minutes of collection.

Eight cats, 18 weeks after FIV infection, and 8 age-matched (30 weeks old), SPF, FIV-negative cats were inoculated with *T. gondii* tachyzoites. The cats were anesthetized by an intramuscular injection of 100 mg ketamine hydrochloride, and the right common carotid artery was surgically isolated. One ml of inoculum (10,000 tachyzoites) was injected in the carotid artery through a 27-gauge needle. Intracarotid inoculation, rather than the natural oral route of infection, was used to enhance the development of ocular lesions, which was the original intent of this model.

T. gondii *Serology*

Serum samples from all the cats were collected before and twice weekly after inoculation with *T. gondii* and assayed by ELISA for *T. gondii* antigen,[23] *T. gondii*-containing IgM and IgG immune complexes,[24] and *T. gondii*-specific IgM and IgG antibodies.[18]

Flow Cytometric Analysis of Lymphocyte Subsets

One week before and at various times after inoculation, an EDTA blood sample was collected for a complete blood count and flow cytometric analysis (FACS) of lymphocyte subpopulations using a panel of monoclonal antibodies developed in our laboratory.[19] The plasma was removed, the cells washed twice in phosphate-buffered saline, resuspended, divided equally into six tubes, and monoclonal antibody added. Two tubes received FITC-conjugated anti-cat Ig (KPL, Kirkegaard and Perry Laboratories Inc., Gaithersburg, MD) and biotin-conjugated anti-pan T, two tubes received FITC-conjugated anti-$CD8^+$ antibody and biotin-conjugated anti-$CD4^+$ antibody. Controls consisted of cells incubated with irrelevant mouse monoclonal antibodies conjugated with FITC and biotin and cells incubated in medium without antibody. Cells were incubated for 30 minutes at room temperature, washed in phosphate-buffered saline, phycoerythrin added (to develop the biotin), and incubated again for 30 minutes at room temperature. After another wash, 1 ml of FACS Lysing Solution (Becton Dickinson Immunocytometry Systems, San Jose, CA) was added to each tube. After a 10-minute incubation, cells were washed, the lysing step repeated, and the cells resuspended in 300 µl for FACS analysis. The percentage of positively stained lymphocytes was determined by flow cytometric analysis using a Becton Dickinson FACScan. Absolute lymphocyte counts were determined using a Coulter counter.

Lymphocyte Blastogenesis Assay

Lymphocyte responses to Concavalin A (Con A) and specific *Toxoplasma* antigens were measured as described by Lappin et al.[25] with minor modifications. Briefly, peripheral blood lymphocytes were purified on a 43%/62% discontinuous Percoll gradient[26] and suspended in RPMI media supplemented with 10% fetal bovine serum at a concentration of 1×10^6 cells/ml. 100 µl of cell suspension and 100 µl of media or mitogen were added to each well of a 96-well round- bottomed plate (Costar, Cambridge, MA) and incubated at 37 C. After 72 hours of incubation, cultures were pulsed with 2 µCi/well of [^{3}H]-thymidine, and cellular DNA collected 18 hours later. Tritium incorporation was measured using a β counter (LKB Wallac, Gaithersburg, MD). The nonspecific mitogen used was Con A at a concentration of 10 µg/ml. The *Toxoplasma*-specific antigens consisted of soluble intracellular antigen at 10 µg/

ml, secretory antigen at 160 μg/ml, and host cell antigen from uninfected Vero cells at 80 μg/ml. The preparation and standardization of these antigens in a lymphocyte blastogenesis assay are described by Lappin et al.[25]

Postmortem Examination

Affected animals were euthanized when moribund with an overdose of barbiturate and necropsied. Tissues collected at necropsy were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin stain.

Immunohistochemistry

Formalin-fixed sections of pulmonary tissue from each cat were stained using a previously described *T. gondii*-specific immunohistochemical staining procedure.[21,27] Briefly, polyclonal mouse anti-*T. gondii* IgG was diluted 1:100 in Tris-buffered saline and used as the primary antibody. Conterstaining was done using commercial kits containing biotinylated anti-mouse IgG and streptavidin-alkaline phosphatase (Histomark, KPL) and substrate and contrast stain (Histomark Red, KPL).

Results

FIV Infection and T. gondii *Challenge of Cats*

Eight 12-week-old SPF cats were inoculated with 6×10^5 $TCID_{50}$ of the $NCSU_1$ isolate of FIV. As reported previously,[9] FIV infection caused a reduction in $CD4^+$ lymphocytes and an increase in $CD8^+$ lymphocytes such that by 18 weeks after inoculation, the mean $CD4^+$:$CD8^+$ ratio had dropped from 3.26 ± 0.82 to 0.59 ± 0.36 (Figure 1). At this time, PBMC from all cats were positive for FIV by RT expression using methods previously described.[12] In addition, all cats had antibody to FIV proteins by ELISA (IDEXX) (data not shown). At 18 weeks after FIV infection, all cats were challenged with *T. gondii*. Eight age-matched, FIV antibody negative (by ELISA, data not shown) control cats with normal $CD4^+$:$CD8^+$ ratios (Figure 1) were similarly inoculated with *T. gondii*.

Clinical Response to T. gondii *Challenge*

All cats, including the FIV-infected cats, were clinically normal at the time of *T. gondii* challenge. *T. gondii*-infected cats in the non-FIV inoculated group developed mild anorexia and lethargy on days 9 to 11 after inoculation and developed multifocal chorioretinitis beginning on days 7 to 10 after inoculation, which resolved over a 3- week course. The infection was otherwise subclinical. Four FIV-infected cats challenged with *T. gondii* developed fever (39.7–41.1 C), depression, and moderate to severe ocular lesions, including chorioretinitis with subretinal granuloma formation, localized retinal detachment, and fibrinous anterior uveitis, beginning on days 8 to 10 after inoculation. Severe and progressive tachypnea, dyspnea, and tachycardia were noted on days 9 to 14 after inoculation, and interstitial and consolidated lung sounds were auscultated. Cats were euthanized when moribund and mortality in the group was 75% (3/4 cats) between days 12 to 15 after inoculation, despite supportive care with subcutaneous fluids, and, in one case, oxygen therapy. The one surviving cat maintained a high fever until day 24 after inoculation and slowly recovered clinically normal pulmonary function. In four additional FIV-inoculated cats infected with *T. gondii,* oral clindamycin hydrochloride (AquaDrops, The UpJohn Company, Kalamazoo, MI), an antiprotozoal antibiotic, at a dose of 12.5 mg/kg twice daily was initiated on the first day of fever (day 9 after inoculation) in an attempt to prevent mortality. Despite therapy, these cats showed a clinical course similar to the untreated FIV-*T. gondii* infected cats described above and had a mortality of 75% (3/4 cats) on days 9 to 11 after inoculation. One cat, in addition to respiratory disease, developed progressive

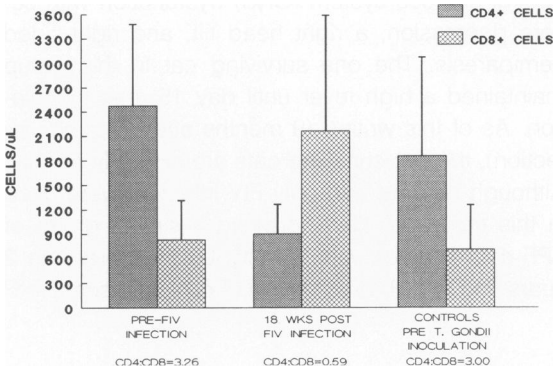

Figure 1. *Mean $CD4^+$ and $CD8^+$ cell numbers of 8 SPF cats before (first two histograms) and 18 weeks after FIV infection but before T. gondii infection (second two histograms), and of 8 age-matched SPF cats before T. gondii infection (last two histograms). Cell numbers were determined by multiplying the percent positive $CD4^+$ or $CD8^+$ cells, determined by flow cytometric analysis, by the total lymphocyte count from a complete blood count (drawn at the same time as the sample for flow cytometry). Error bars represent standard deviations of the means. The $CD4^+$:$CD8^+$ ratios represent the means of the individual ratios.*

central nervous system (CNS) dysfunction with severe depression, a right head tilt, and right-sided hemiparesis. The one surviving cat in this group maintained a high fever until day 15 after inoculation. As of this writing (9 months after *T. gondii* infection), the two surviving cats are clinically normal. Although no cats with only FIV infection were used in this study, we have followed a similar group of SPF cats infected with FIV-$NCSU_1$ for more than 2 years and found no evidence of clinical disease.[9,20]

T. gondii *Serology*

All 16 cats developed detectable levels of *T. gondii*-specific IgM, most by 4 days after inoculation. By 14 days after inoculation, 3 of the 8 *T. gondii*-only cats were positive for *T. gondii*-specific IgG and 7 of 8 were positive by 18 days after inoculation. Only 4 of the FIV-*T. gondii* cats survived until day 14 after inoculation, and none had detectable *T. gondii*-specific IgG. At 18 days after inoculation, 1 of the 2 surviving *FIV-T. gondii* cats was positive for *T. gondii*-specific IgG, and both surviving cats ultimately developed IgG titers of similar magnitude to the *T. gondii*-only cats. *T. gondii*-specific antigens and immune complexes were detected in the serum in both groups of cats and the levels were not significantly different between the two groups.

Lymphocyte Changes

Infection of cats with the $NCSU_1$ isolate of FIV caused a rapid (4 weeks) inversion in the $CD4^+$:$CD8^+$ ratio characterized by a decrease in $CD4^+$ cell numbers and an increase in $CD8^+$ cell numbers. At the time of *T. gondii* infection (18 weeks after FIV infection), the mean $CD4^+$:$CD8^+$ ratio of the 8 cats was 0.59 (Figure 1). In contrast, the mean $CD4^+$:$CD8^+$ ratio of the age-matched controls was 3.0 (Figures 1 and 2), well within the reported values for young adult SPF cats.[9] After infection with *T. gondii*, the control cats (non-FIV) developed a panlymphopenia ($CD4^+$, $CD8^+$ and B cells) at 1 week after inoculation followed by a gradual recovery (2–4 weeks) of the $CD4^+$ and $CD8^+$ cells, and a dramatic B cell lymphocytosis peaking at 4 weeks after inoculation (Figure 2). At 8 weeks after inoculation, these cats had normal $CD4^+$:$CD8^+$ ratios (≈2:1) for cats exposed to a variety of (non-FIV) infectious agents.[12] By 15 weeks after inoculation, cell numbers had returned to pre-*T. gondii* infection levels.

The FIV-infected *T. gondii*-challenged cats also developed a panlymphopenia ($CD4^+$, $CD8^+$, and B cells) at 1 week after inoculation (Figure 3). However, lymphocyte numbers continued to decrease after 1 week after inoculation in 6 of the 8 cats. These 6 cats were euthanized between days 9 and 15 after inoculation, and no other data were available (Figure 3A). Of the two cats that recovered from the *T. gondii* challenge, the $CD4^+$ and $CD8^+$ cells gradually increased beyond pre-*T. gondii* numbers in a pattern typical of FIV infection in that there was a greater increase in $CD8^+$ cells than in $CD4^+$ cells. However, this increase in lymphocyte numbers was not sustained and by 15 weeks after inoculation, both $CD4^+$ and $CD8^+$ cells had returned to pre-*T. gondii* infection levels (Figure 3B). Interestingly, the two cats that survived the infection had the lowest number of $CD8^+$ cells at the time of challenge (Figure 3B). The B cell response in these two cats was similar to that of the *T. gondii* only cats, with a dramatic B cell lymphocytosis peaking at 4 weeks after inoculation, then a decrease to preinfection levels by 15 weeks after inoculation (Figure 3B).

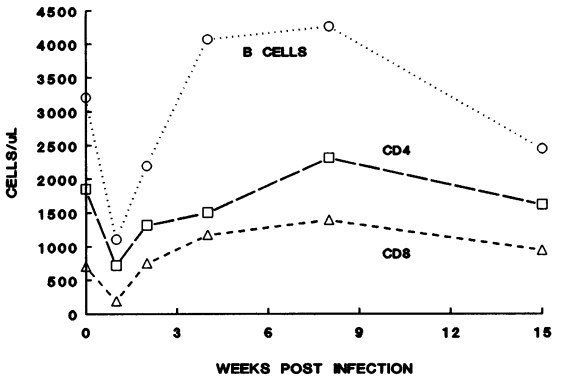

Figure 2. *Mean $CD4^+$, $CD8^+$, and B cell numbers of 8 FIV-negative cats inoculated via the carotid artery with T. gondii tachyzoites. Cell numbers were determined as described in Figure 1. Inoculated cats experienced an early (1 week) panlymphopenia, then gradual recovery of cell numbers to preinoculation levels.*

Lymphocyte Response to Mitogens

Lymphocyte responses to a T cell mitogen (Con A), two *T. gondii* antigens, and a host cell antigen were evaluated in 4 cats from each group at 1 week after inoculation, and 4 *T. gondii* only and the 2 remaining FIV-*T. gondii* cats at 4 and 6 weeks after inoculation. Unfortunately, due to the acute, fatal course of the dual infection, meaningful comparisons of mitogen responses cannot be made. However, two important points can be noted. At 1 week after *T. gondii* challenge, the FIV/*T. gondii* cats and 3 of the 4 *T. gondii*-only cats had suppressed mitogenic response to Con A when compared with normal uninfected controls (Table 1). Second, although there

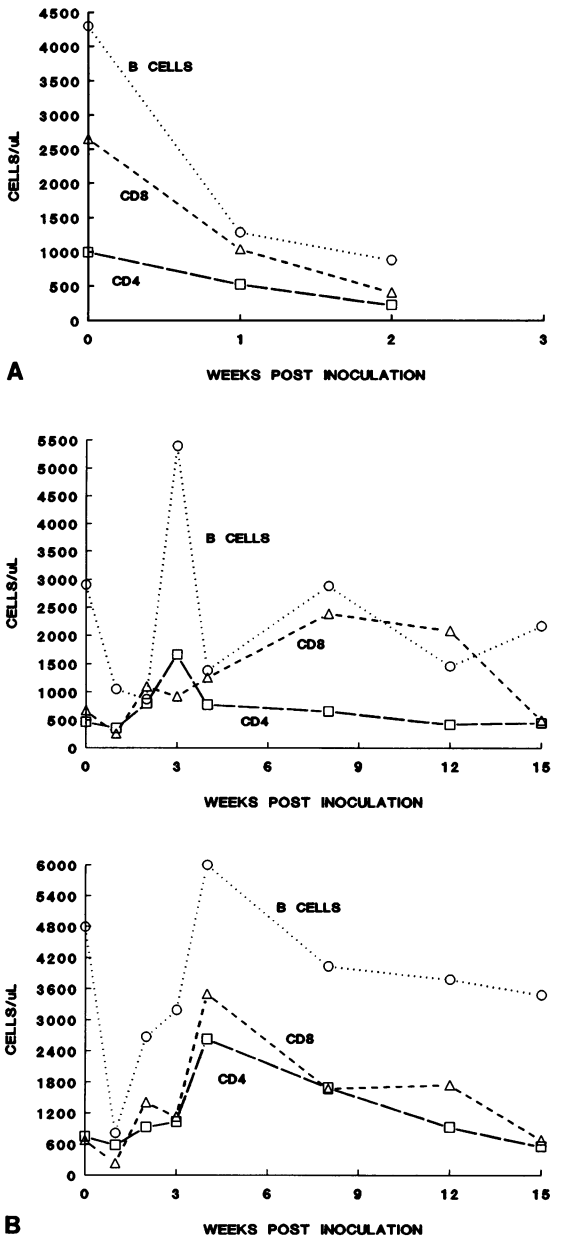

Figure 3. *Lymphocyte changes in FIV-infected cats after inoculation via the carotid artery with T. gondii tachyzoites. A: Mean CD4+, CD8+, and B cell numbers of 6 cats that developed severe interstitial pneumonia and were euthanized 2 weeks after inoculation. B: The CD4+, CD8+, and B cell numbers of the 2 cats (each graph represents a single cat) that recovered from T. gondii infection. Both groups of cats developed a panlymphopenia 1 week after inoculation. Lymphocytes in the two cats that survived then gradually increased in number, while cell numbers in the cats that were euthanized continued to drop.*

were no significant responses by any of the cats to *T. gondii* antigens (data not shown), the unstimulated baseline counts of the 3 *T. gondii*-only infected cats was higher than either the dual infected cats or uninfected control cats. This suggests that the *T. gondii*-only cats were responding *in vivo* to *T. gondii*

Table 1. *Proliferative Response to Con A of Peripheral Blood Lymphocytes Collected 1 Week after Infection with T. gondii*

Treatment group	Unstimulated*	Con A-stimulated*
Normal control	433†	66030
	244	70677
T. gondii only	4193	5867
	1953	15116
	1010	7339
	521	25641
FIV-*T. gondii*	591	8434
	317	6136
	479	9166
	286	5148

* 1 × 10^6/ml Percoll purified PBMC were incubated for 72 hours in medium without mitogens (unstimulated) or with 10 μg/ml of Con A (stimulated) as described in Materials and Methods, pulsed with tritium, and harvested 18 hours later.

† Numbers represent the mean counts per minute of triplicate samples from individual cats within each treatment group.

antigens while the FIV-*T. gondii* cats were not (Table 1). The fourth *T. gondii*-only cat was similar in its response to both Con A and the antigens as the controls (Table 1). Both the *T. gondii*-only cats and the 2 surviving dually infected cats responded to *Toxoplasma*-specific antigens at 4 and 6 weeks after inoculation and had Con A responses similar to uninfected control cats (data not shown).

Postmortem Findings

Gross Findings

The lungs of affected animals (6/6 cats) were mottled red-tan-brown, firm, and did not collapse as the thorax was opened. Touch imprints of cut surfaces revealed macrophages, some with intracellular tachyzoites, scattered extracellular tachyzoites, lesser numbers of neutrophils, lymphocytes, pulmonary epithelial cells, and cell debris. Livers of affected animals were normal in size to slightly enlarged, and mottled with multiple pale, tan, 1- to 3-mm foci of discoloration consistent with hepatic necrosis. In addition, the hearts of affected animals contained occasional foci of myocardial necrosis characterized by pale white streaks on epicardial and cut surfaces. All animals had generalized lymphadenomegaly.

Microscopic Findings

The most severe microscopic lesions were consistently present in the lungs and liver. Microscopic examination of the lungs revealed locally extensive to diffuse interstitial pneumonia with focal areas of

necrosis (Figure 4A). Alveolar septae were congested and expanded by macrophages, neutrophils, lesser numbers of lymphocytes, occasional plasma cells, and eosinophils. Alveoli were lined by prominent type II pneumocytes and partially filled with a similar population of inflammatory cells, red blood cells, cell debris, and small amounts of protein and fibrin (Figure 4B). Occasional multinucleate macrophages, epithelioid macrophages, and rare mitotic figures were also present within alveolar lumina. Foci of necrosis were consistently located in areas of the most intense inflammation and were characterized by focal loss of septal architecture, accumulation of neutrophils, macrophages, red blood cells, fibrin, and cell debris. *T. gondii* tachyzoites were most numerous in areas of necrosis, usually intracytoplasmic within alveolar macrophages and neutrophils, but occasionally extracellular, admixed with the alveolar cell debris. Tachyzoites were rarely seen intracytoplasmic within bronchial or bronchiolar epithelial cells adjacent to the most severe lesions.

In addition to interstitial pneumonia, 1 of 6 cats had focal to focally extensive areas of suppurative bronchopneumonia characterized by accumulation of neutrophils and cell debris in the lumina of alveoli and bronchioles. Intralesional bacteria were occasionally observed in these areas.

All animals had multifocal to coalescing hepatic necrosis, which was frequently quite severe. Within necrotic foci, the liver architecture was effaced by various combinations of lymphocytes, macrophages, red blood cells, neutrophils, fibrin, and cellular debris. Portal areas were also expanded by large numbers of lymphocytes and lesser numbers of macrophages. *T. gondii* tachyzoites were occasionally observed in necrotic foci. The majority of tachyzoites were intracytoplasmic within macro-

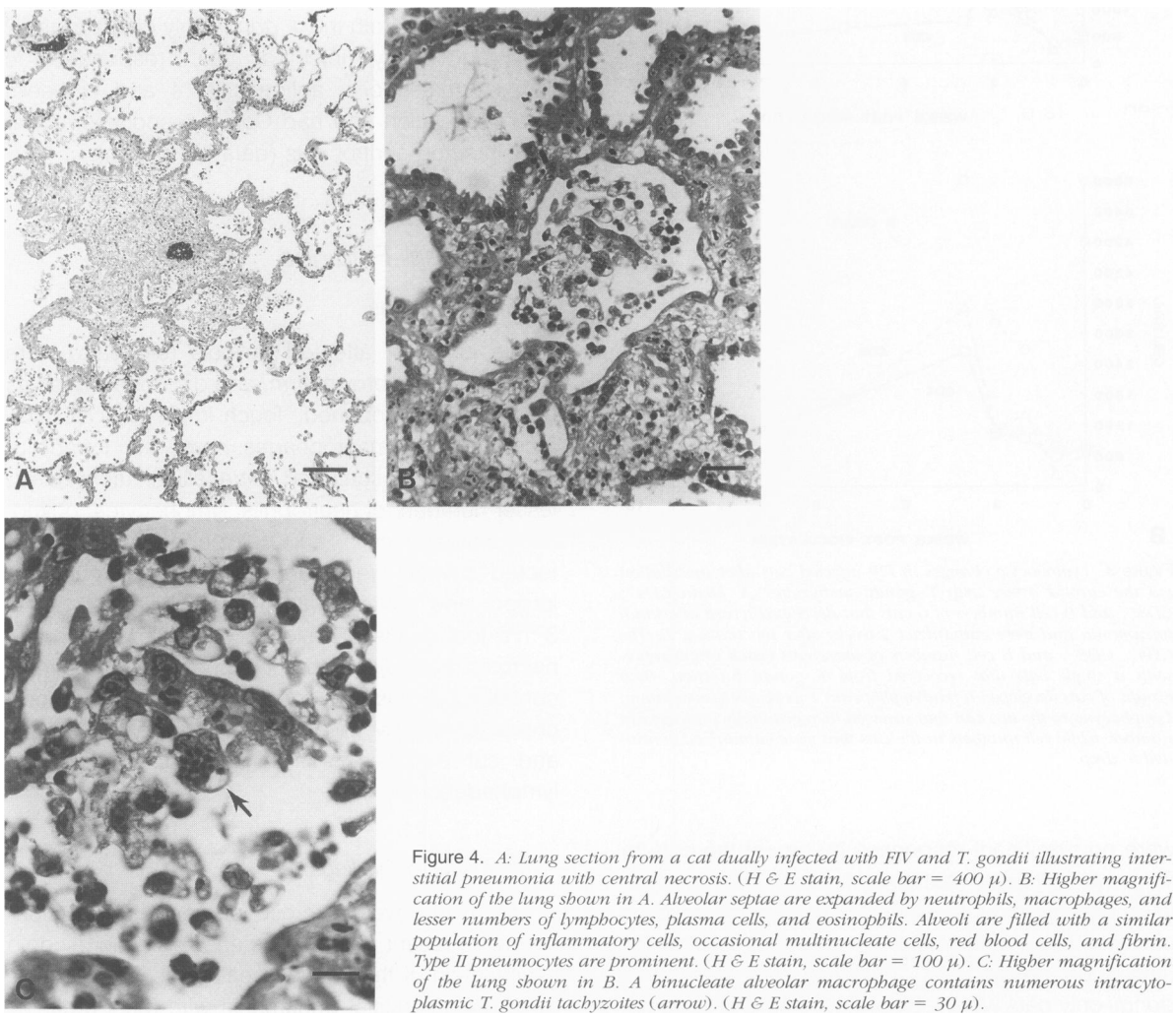

Figure 4. *A: Lung section from a cat dually infected with FIV and T. gondii illustrating interstitial pneumonia with central necrosis. (H & E stain, scale bar = 400 μ). B: Higher magnification of the lung shown in A. Alveolar septae are expanded by neutrophils, macrophages, and lesser numbers of lymphocytes, plasma cells, and eosinophils. Alveoli are filled with a similar population of inflammatory cells, occasional multinucleate cells, red blood cells, and fibrin. Type II pneumocytes are prominent. (H & E stain, scale bar = 100 μ). C: Higher magnification of the lung shown in B. A binucleate alveolar macrophage contains numerous intracytoplasmic T. gondii tachyzoites (arrow). (H & E stain, scale bar = 30 μ).*

phages, but occasional free tachyzoites were admixed with cell debris in these foci. Additionally, hepatocytes along the edges of necrotic foci occasionally contained intracytoplasmic tachyzoites.

Less severe lesions were present in other tissues. Mild lymphocytic myocarditis with focal areas of necrosis were seen in all 6 cats. Mild, focal interstitial nephritis was also commonly seen in all the cats.

Affected animals also had multifocal, non-suppurative meningoencephalitis. Lesions were most prominent in the cerebral gray matter, but were also present in the cerebellum and brain stem. In affected areas, the meninges and spaces of Virchow-Robin were focally expanded by mononuclear cells and rare neutrophils, which extended into the adjacent neuropil for a short distance. The adjacent neuropil was often necrotic, characterized by loss of tissue architecture, increased numbers of astrocytes and microglia, fibrin, red blood cells, and accumulation of inflammatory cells and neuronal cell debris. Occasional free tachyzoites were present in necrotic foci. Neurons within inflammatory foci occasionally contained *T. gondii* tissue cysts. Lesions in the cerebellum and brain stem were generally milder and consisted of rare, randomly scattered nodules of microglia in the gray and white matter. The spinal cord was not examined.

Animals (6/6 cats) also had mild ocular lesions. The most consistent lesion was minimal to mild, non-suppurative anterior uveitis with rare, focal necrosis. Inflammation was most prominent at the iris at the iridocorneal angle. One animal had a lesion in the posterior choroid adjacent to the optic nerve. Blood vessels in the sclera and, to a lesser extent, uvea were often surrounded by a thin band of lymphocytes. Tachyzoites were not seen in the ocular lesions.

The mesenteric lymph nodes of all 6 cats contained prominent germinal centers, and an increased number of macrophages in the subcapsular sinus and medulla. The spleen contained prominent germinal centers, and the red pulp was diffusely infiltrated with macrophages. Organisms were rarely identified in these locations. When present, organisms were located intracytoplasmic within macrophages. Peripheral lymph nodes were not examined.

Multiple sections of small and large intestine were reviewed and considered microscopically normal. Small, random foci of pancreatic necrosis were also seen in 3 of the cats.

The gross and microscopic lesions were consistent with severe, disseminated toxoplasmosis.

Immunohistochemistry

Formalin-fixed sections of lung from each cat were stained for the presence of *T. gondii* antigen using an immunohistochemical staining procedure. Tachyzoites were most numerous in areas of severe interstitial pneumonia and necrosis. In these foci, organisms were seen within macrophages, multinucleate giant cells, neutrophils, and occasionally, type II alveolar epithelium, bronchiolar epithelium, or rarely, bronchial epithelium. Rarely, individual tachyzoites were seen within neutrophils and/or macrophages in alveolar septae of unaffected areas of lung.

Discussion

There is evidence that HIV-1-infected individuals develop $CD4^+$ T cell dysfunction early after infection that precedes the quantitative reduction of the $CD4^+$ lymphocyte population.[28,29] It has been speculated that this early $CD4^+$ dysfunction in HIV infection is a selective failure to proliferate in response to certain antigens (anergy) or a depletion of antigen-specific $CD4^+$ clones. Thus, selective depletion of $CD4^+$ clones to such agents as *T. gondii* could not only lead to reactivation of latent infection late in HIV infection, but could also predispose an individual to a more severe primary *T. gondii* infection relatively early after HIV infection. To better understand the complex immunological factors contributing to the pathogenesis of these interactive infections, experiments were designed to explore possible effects of a pre-existing FIV infection on the response to a primary avirulent *T. gondii* infection in the cat.

As predicted from previous studies,[21] intracarotid inoculation of normal cats with *T. gondii* caused multifocal chorioretinitis, but an otherwise subclinical infection. Hematologically, the infection was characterized by a transient panlymphopenia followed by recovery of all lymphocyte subsets to near or above normal preinfection levels. This is a characteristic response to many infectious agents and is indicative of a normal immune response to the pathogen.[1,9,30] In contrast to the *T. gondii*-only infected cats, *T. gondii* infection of the FIV-infected cats caused an acute systemic disease in all animals, resulting in euthanasia of 6 of 8 cats between 10 and 14 days after inoculation. Clinically, the cats were febrile, demonstrated severe respiratory distress, and in one case, CNS signs. The lung lesions of all 6 euthanized cats were characterized by severe interstitial inflammation and infiltration of mac-

rophages, many of which morphologically were similar to activated macrophages. Sections of lung stained with hematoxylin and a *T. gondii*-specific immunohistochemical technique demonstrated that tachyzoites were most numerous in areas of severe interstitial pneumonia and necrosis. In addition to the lung, we found lesions consistent with generalized toxoplasmosis in the liver, spleen, mesenteric lymph nodes, heart, kidney, eye, and CNS. These postmortem findings are similar, with a few exceptions, to those described previously by Parker et al.[31] in SPF cats infected intravenously via the jugular vein with the RH strain of *T. gondii*. In that study, lesions were not identified in the eye, were rare in the kidney (1/17 cats), and were milder in the brain than we describe in our study. In addition, reactivation of latent *T. gondii* in the cat by immunosuppressive doses of corticosteroids led to clinical disease and postmortem findings very similar to those described herein, especially interstitial pneumonia.[15] This is in contrast to reactivation of latent *T. gondii* in humans, where CNS signs are the major clinical presentation. Interestingly, mice infected with the AIDS-inducing LPBM5 murine leukemia virus (MAIDS) and challenged with the ME49 strain of *T. gondii* developed an acute form to toxoplasmosis similar to that described herein, including interstitial pneumonitis, myocarditis, and hepatic necrosis.[32]

Our results differ quantitatively from those reported by Lin et al.[33] Lin et al.[33] reported that concurrent infection of cats with FIV and *T. gondii* (inoculated orally) appeared to favor *T. gondii* replication and enhance suppression to mitogens as compared with infection with either agent alone. These changes, however, were mild as compared with the marked effects described herein. In contrast to our observation, Lin et al.[33] reported that FIV did not cause an increase in $CD8^+$ cells. It is possible that differences in the two studies are due to the use of different isolates of FIV or different isolates and routes of administration (oral versus parenteral) of *T. gondii*. In addition, the FIV infected cats in this study had reduced $CD4^+$ numbers and inverted $CD4^+$:$CD8^+$ ratios at the time of *T. gondii* challenge, which may have increased susceptibility to *T. gondii* challenge. Additional studies will be necessary to answer these questions.

Although we did not evaluate the mitogen response or IL-2 production of the FIV-infected cats before *T. gondii* infection, previous studies predict that they would have been relatively normal.[10,34,35] These cats had relatively early stage FIV infections, and it has been reported that, as in early stage HIV-1 infection, these cats respond normally to mitogens and produce normal amounts of IL-2.[10,34] However, the absence of an appropriate immune response in the dually infected cats is suggested by the absence of an IgG response and the absence of an *in vivo* proliferative response (^3H uptake by PBMC) to *T. gondii* antigens at 2 weeks after *T. gondii* challenge, whereas both immune response parameters were positive for the *T. gondii*-only infected cats.

How FIV infection predisposes cats to acute toxoplasmosis is not known. There is considerable evidence that protective immunity to *T. gondii* is cell-mediated. Adoptive transfer studies in mice, rats, and guinea pigs have demonstrated that resistance is dependent on the presence of T lymphocytes, and particularly $CD8^+$ cells.[36–38] The other major mechanism of defense is the activated macrophage.[39–41] Both of these defense mechanisms depend on the $CD4^+$ cell and its cytokines for activation, and thus decreased $CD4^+$ numbers or function could result in uncontrolled replication of *T. gondii*. Indeed, the importance of $CD4^+$ cell numbers in the control of *T. gondii* infection is supported by studies demonstrating increased mortality from *T. gondii* pneumonia[42] and increased tissue cyst formation in brains[43] in $CD4^+$-depleted mice.

The major cytokine involved in control of *T. gondii* is interferon-γ (IFN-γ), which is a principal mediator of macrophage activation.[44,45] Suzuki et al.[41,46] demonstrated that administration of antibody against IFN-γ predisposed mice to acute, fatal toxoplasmosis and that susceptibility correlated with failure to generate both activated macrophages and $CD8^+$ cell-mediated resistance. Interestingly, the acute toxoplasmosis, with lesions similar to those described herein, that developed in the MAIDS mice described by Gazzinelli et al.[32] was associated with reduced ability to produce IFN-γ.

T. gondii infections secondary to HIV are usually the result of reactivation of latent *T. gondii* tissue cysts and are characteristically manifested as focal or diffuse meningoencephalitis.[6,47] While there was clinical and histological evidence of CNS involvement, including early formation of *T. gondii* tissue cysts, in dually FIV-*T. gondii* infected cats in this study, the predominant clinical and microscopic lesions were interstitial pneumonia and hepatic necrosis, characteristic of acute toxoplasmosis. These differences may be host-related, as the lung appears to be the major target in both primary[31] and reactivated[15] toxoplasmosis in cats. However, it should be noted that in HIV-infected individuals, primary exposure to *T. gondii* can result in disseminated disease including pneumonia, myocarditis,

and myositis, as well as CNS involvement,[48] suggesting that the clinical disease depends on whether primary *T. gondii* infection occurs before or after infection with HIV. This is supported by the observations in the murine MAIDS model, where primary exposure to *T. gondii* resulted in acute, generalized toxoplasmosis, whereas reactivation of chronic *T. gondii* by MAIDS was characterized by classic CNS encephalitis.[32] In addition, lesions similar to those described herein have been described in nude mice[49] and in SCID mice[50] after primary *T. gondii* infection.

It is important to confirm the differences between acute primary toxoplasmosis and reactivation toxoplasmosis in the feline/FIV model. To this end, we have recently challenged *T. gondii* chronically infected cats with FIV. Although we have established FIV infections as indicated by FIV provirus in PBMC, antibody response to FIV antigens, and inverted $CD4^+$:$CD8^+$ ratios in all the cats, we have no evidence of *T. gondii* reactivation as of this writing (6 months after FIV challenge). It will be of interest to monitor these cats further to determine whether *T. gondii* is reactivated and where the major lesions develop.

Whatever the mechanism by which FIV predisposes cats to acute generalized toxoplasmosis and life-threatening respiratory impairment, this dual infection provides an excellent model to study lentivirus-induced immune dysfunction. Although the model as described does not address mechanisms of reactivation of latent opportunistic infections at late stages of HIV infection, it should provide an excellent system to address the targets of the immune dysfunction identified early after HIV infection[28,29] and how these defects may relate to progressive loss of $CD4^+$ cells or $CD4^+$ cell function and the ultimate loss of resistance to opportunistic infections.

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