

Pathological findings in a cat with cryptococcosis and feline immunodeficiency virus infection

J.A. Ramos-Vara, L. Ferrer and J. Visa

Faculty of Veterinary Medicine, Autonomous University, Bellaterra, Barcelona, Spain

Summary. This report describes the gross, histopathological, immunocytochemical and electron microscopic findings in a cat with systemic cryptococcosis and feline immunodeficiency virus (FIV) infection. Lymphadenopathy and cloudiness of leptomeninges were the major gross findings. Numerous cryptococcal yeasts were found in lymph nodes, brain, and lung, and were less common in the kidney and the eye. The inflammatory reaction varied in intensity and cell type (mononuclear through granulomatous) depending on the organ involved. Yeasts were mainly within phagocytes as revealed by electron microscopy. Some inflammatory cells were immunocytochemically-stained with anti-CD3 antibodies.

Key words: Cat, *Cryptococcus neoformans*, Feline immunodeficiency virus

Introduction

Cryptococcosis is uncommon in small domestic animals, but is the most common systemic fungal disease in the cat (Legendre, 1989). The organism usually colonizes the upper respiratory tract and eventually the lungs. From the lungs this fungus can disseminate to the eyes, central nervous system and skin. Feline leukemia virus predisposes to cryptococcosis, probably due to immunosuppression observed in this disease (Legendre, 1989) as has been described in human beings with diseases such as AIDS that impair immunity (Huffnagle and Lipscomb, 1992). Other immunosuppressive factors in the cat probably predispose to developing this fungal infection. In this report we describe a case of systemic cryptococcosis in a cat with feline immunodeficiency virus (FIV) infection.

Materials and methods

In the last four years we have received in our diagnostic service several cases of cryptococcal infections in small animals. Three were in cats and one in a dog. Two cats were FeLV negative and FIV positive; the other was both FeLV and FIV negative. One of the cats (Siamese, male, 5 years old) was put down and submitted for necropsy because of the poor prognosis; it was FeLV negative and FIV positive (CITE Combo R, Portland, USA).

The clinical study of this case and others has been previously described (Ferrer et al., 1992b). At necropsy, samples of multiple organs were taken immediately and fixed in 10% neutral-buffered formalin and eventually embedded in paraffin. Some of the formalin-fixed samples from brain and lung were processed for electron microscopic studies. Immunocytochemistry was used to detect CD3 antigen following a previously described technique (Ferrer et al., 1992a). Briefly, deparaffined sections were treated with pronase (0.1% in tris-buffered saline) for 10 minutes at room temperature (rt). The immunocytochemical technique used was the peroxidase-antiperoxidase method (PAP) using a rabbit antiserum against human CD3 molecule, incubated at 4 °C for 18 h and then goat anti-rabbit IgG antiserum (30 min, rt) and PAP complex (30 min, rt). The reaction was developed with DAB as chromogen. Lymphoid tissues from normal cats were used as controls.

Results

Gross findings included severe enlargement of cervical lymph nodes, which were pale, firm and homogeneous on cut surface. There was a patchy cloudiness of the leptomeninges. Small (1-2 mm in diameter), grey spherical foci were scattered throughout the cortex, midbrain, cerebellum, and spinal cord. Lungs were rubbery in consistency.

Histopathological study of affected lymph nodes revealed a granulomatous reaction that involved most of

the lymphoid tissue (Fig. 1). The subcapsular sinus was filled with cryptococcal organisms. The grey foci noted grossly in the brain were cryptococcal cysts, mostly intact, with little or no peripheral mononuclear (lymphocyte-like) cell infiltration. Leptomeninges had numerous cryptococci with a mild inflammatory reaction. Some of these cells immunocytochemically stained for CD3 marker (a specific marker for T-lymphocytes in several species). The lung was affected by granulomatous interstitial pneumonia with epithelioid cells surrounding cryptococcal yeasts (Fig. 2). Most organisms in the lung were degenerated as was confirmed by electron microscopy (Fig. 3) and were free

or within phagocytic cells (Fig. 4). Other organs, such as the eye and kidneys, had cryptococcal yeasts with moderate inflammation. Skin lesions were not found.

Discussion

We describe in this report morphological studies of a cat with systemic cryptococcosis and FIV infection. Cryptococcosis in the North of Spain is uncommon. In this cat, gross and microscopic lesions were similar to those described by other authors (Kalina et al., 1974; Wilkinson, 1979). The inflammatory reaction against cryptococcal organisms varied, depending on the organ

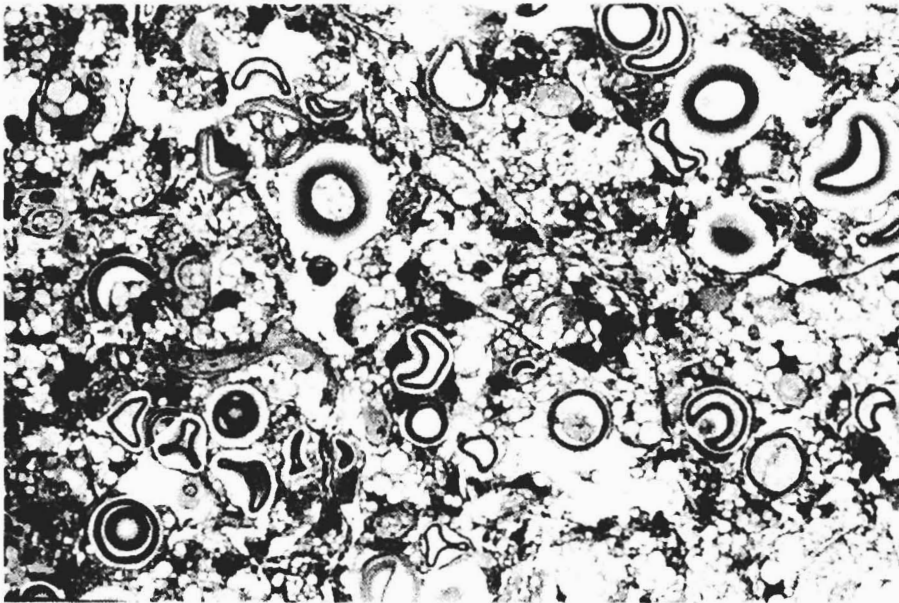


Fig. 1. Cervical lymph node. Numerous cryptococcal forms, some of them apparently intact, can be seen free or within phagocytic cells. Toluidine blue. x 640

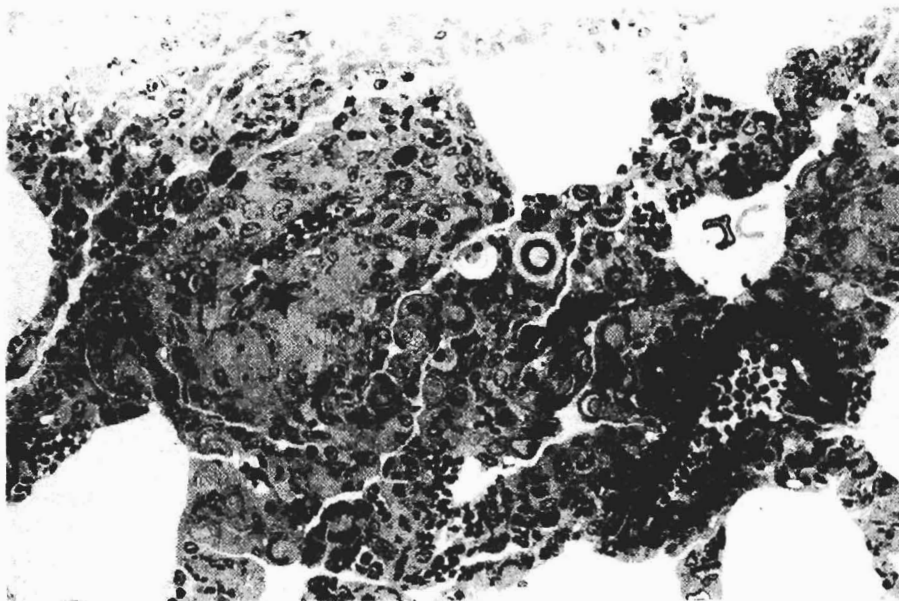


Fig. 2. Lung. Granulomatous pneumonia. Numerous epithelial cells (star) and cryptococcal organisms in different stages of degeneration can be seen. Toluidine blue. x 400

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involved: it was mild or absent in the CNS and intense in the lungs and affected lymph nodes. FIV infects both CD4 and CD8 T-lymphocytes (Brown et al., 1991), thereby affecting cellular-mediated immunity. Secondary infections, such as cryptococcosis, are not uncommon in these immunocompromised animals as can be expected, because T-cell-mediated immunity is the most important component of host defences against *Cryptococcus neoformans* (Huffnagle and Lipscomb, 1992). CD4 T-cell inflammatory response is claimed to prevent the dissemination of *C. neoformans* from the lungs to other parts of the body (Hill, 1992). Probably, in our case, CD4 T-lymphocyte immune activity was altered by

concurrent FIV infection, enabling cryptococci to disseminate. The pattern of inflammatory response in our case resembled that observed in T-cell deficient nu/nu mice experimentally infected with *C. neoformans* (Salkowski and Balish, 1991) although in our case the skin was spared and the predominant inflammatory cell was the macrophage rather than the neutrophil. Probably, the degree of immunodeficiency of the cat and the antifungal treatment modified the inflammatory response.

Having used as tissue control feline normal lymph nodes, we also describe in this report for the first time the use of a polyclonal CD3 antiserum that specifically

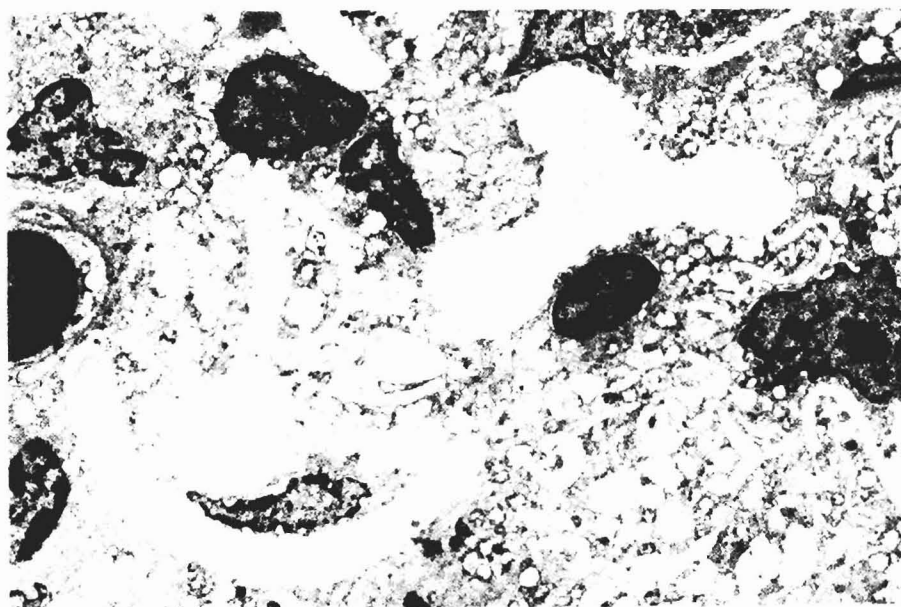


Fig. 3. Lung. Several cryptococcal forms are being lysed by phagocytic cells. x 5,000

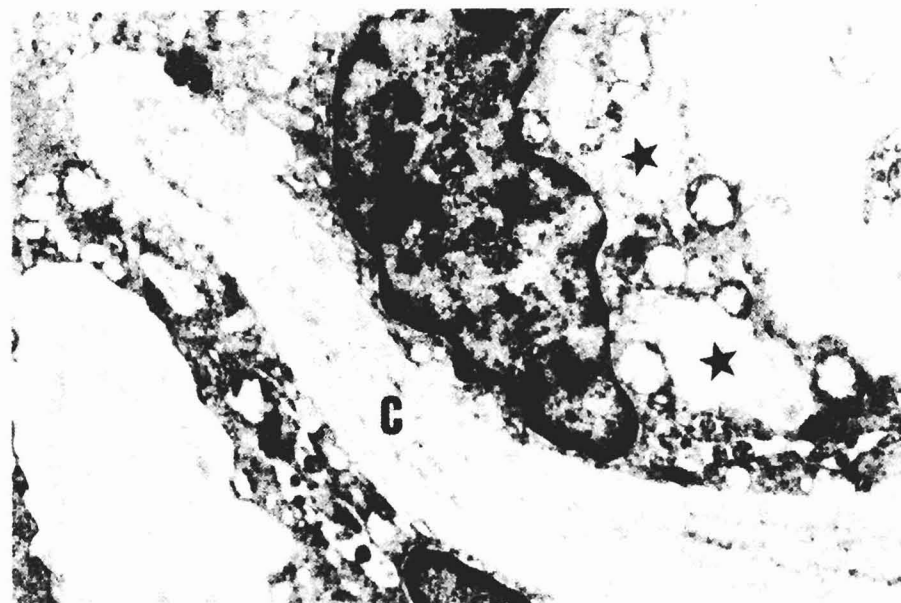


Fig. 4. Cervical lymph node. Several cryptococci (C), almost disintegrated, are encircled by phagocytic cells. Notice that some phagocytic vesicles (star) contain material of a similar appearance to that belonging to cryptococci. x 14,000

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stains T-lymphocytes in routinely-processed feline tissues for histopathology, as previously demonstrated in human (Mason et al., 1989) and dog (Ferrer et al., 1992a) lymphoid tissues. The presence of CD3 lymphocytes in the inflammatory infiltrate indicates an immune-mediated response to the fungus, but we do not know whether this response was protective. Cryptococcus infection in FIV-positive cats might be a useful model to study the pathogenesis of this infection in human beings due to the similarities of FIV and AIDS (Pedersen et al., 1989).

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