T-cell-rich B-cell Lymphoma in the Cat

M. J. Day, M. Kyaw-Tanner*, M. A. Silkstone†, V. M. Lucke and W. F. Robinson*

Department of Pathology and Microbiology, University of Bristol, Langford BS40 5DU, United Kingdom, *Department of Veterinary Pathology and Anatomy, School of Veterinary Science and Animal Production, University of Queensland, Brisbane, Queensland 4072, Australia and †Abbey Veterinary Services, 14 Oak Place, Newton Abbot, Devon TQ12 2HW, United Kingdom

Summary
The clinical and pathological features of eight cases of feline T-cell-rich B-cell lymphoma are described. The disease occurred in older cats (mean age 11.4 ± 3.9 years), which on initial examination generally showed enlargement of a single submandibular or cervical lymph node. After excision, there was no recurrence of the lesions at 6 months in three cats. In one further case, however, the lesion had recurred 6 months later; it was again excised but recurred after an additional 6 months. Microscopically, there was effacement of normal lymph node architecture by a nodular (n = 4) or diffuse (n = 4) proliferation of small to blastic lymphocytes, accompanied by a characteristic population of bizarre giant, or multinucleate, cells. The mitotic rate was low and mitoses were restricted to the atypical population. Immunophenotyping revealed the smaller lymphocytes to be a mixture of CD3+ MHC Class II+ T lymphocytes and BLA36+CD79variable MHC Class IIvariable B lymphocytes. The atypical cells were of the B-cell lineage (BLA36+MHC Class IIvariable). Polymerase chain reaction analysis revealed no proviral DNA products of feline leukaemia virus or feline immunodeficiency virus in tissue from any tumour, confirming that these neoplasms were not associated with either virus. The clinical, histological and immunophenotypic findings in these cats were identical with those of “nodular lymphocyte predominance (lymphocytic and histiocytic/L&H) Hodgkin’s disease” in man.

Introduction
Lymphoma in the cat is generally associated with viraemic or latent infection by the feline leukaemia virus (FeLV), with well-documented molecular pathogenesis (Rezanka et al., 1992). FeLV provirus may be demonstrated in tumour tissue in the absence of viraemia or immunohistochemically-identified viral antigen (Jackson et al., 1996); however some FeLV-negative cats with lymphoma do not have lesional provirus (Hartmann et al., 1998). Additionally, a role for feline immunodeficiency virus (FIV) in the development of some of these tumours has been proposed (Gallanan et al., 1996; Endo et al., 1997). A spectrum of anatomical forms of this neoplasm is recognized in the cat, but multicentric, alimentary and thymic lymphomas are the commonest (Valli, 1993). The microscopical appearance of feline lymphoma is generally one of diffuse infiltration by uniform T or B lymphoid cells, with a high proportion...
of immunoblastic tumours as classified by the National Cancer Institute Working Formulation (Valli, 1993; Jackson et al., 1996).

In addition to overt lymphoma, other distinct syndromes of idiopathic lymphadenopathy are documented in the cat. A series of cats (aged 5 months–2 years) with lymphadenopathy, characterized by distorted lymph node microarchitecture (paracortical proliferation of mixed macrophages, lymphocytes, immunoblasts and plasma cells), was reported by Moore et al. (1986). Most of these cats were positive for FeLV and one subsequently developed lymphoma. By contrast, in a second series of cats (aged 1–4 years) with generalized lymphadenopathy, characterized by paracortical expansion by uniform mitotic lymphocytes with abnormal follicular aggregates, there was no evidence of FeLV infection and the lymphadenopathy resolved over a 12–18 month period (Mooney et al., 1987).

Recently, a case history of a 13-year-old, domestic short-haired cat with a recurrent single mass in the parotid region was reported (Steele et al., 1997). The microscopical appearance of this tumour was one of scattered atypical giant cells within a uniform background of small lymphocytes. On immunohistochemical examination, the atypical population expressed a B-lymphocyte marker and the small lymphocytes largely expressed the T-cell marker CD3. These features were considered consistent with those of human T-cell-rich B-cell lymphoma (Warnke et al., 1995).

In this report, we present a further eight cases of this tumour in the cat. The disease is characterized by the development of a single mass in the submandibular to ventral cervical region of older cats. The microscopical features are those of scattered, atypical giant B lymphocytes within a background of mixed T and B lymphocytes. The tumour is not associated with FeLV infection, and is possibly unassociated with FIV infection. This feline tumour has clear parallels to the human neoplasm “nodular lymphocyte predominance (lymphocytic and histiocytic/L&H) Hodgkin’s disease”.

Materials and Methods

Case Material

Cases were identified on the basis of histopathological examination of lymph node biopsies submitted to the Comparative Pathology Laboratory, Department of Pathology and Microbiology, University of Bristol (n = 3) or to Abbey Veterinary Services (n = 5). Biopsies were submitted between September 1995 and January 1998. Tissue was submitted in 10% neutral buffered formalin, and haematoxylin and eosin (HE)-stained sections were prepared from each biopsy in a routine manner.

Immunohistochemistry

Sections from each biopsy were labelled with a panel of cross-reactive antisera specific for the pan-T lymphocyte marker CD3, the pan-B lymphocyte and plasma cell marker CD79, the B lymphocyte marker BLA36 (expressed by early and activated B cells and Hodgkin’s cells), immunoglobulin λ or κ light chains, and Class II molecules of the Major Histocompatibility Complex (MHC). Antisera specific for feline IgG (Fc-specific) and feline IgM (Fc-specific) were also used. Negative control sections were incubated with normal mouse, rabbit or goat serum in place of specific antibodies.
The cross-reactive antisera specific for conserved peptide sequences of CD3 and CD79 (Jones et al., 1993) have been validated previously for use in the cat (Day, 1995; Callanan et al., 1996), as have the antisera specific for BLA36 (Steel et al., 1997), MHC Class II (Day, 1998) and the immunoglobulin light chains (Day, 1995).

Sections for immunohistochemistry were pre-treated by passage through graded alcohol and exposure to hydrogen peroxide (0.5%) in methanol to block endogenous peroxide activity. Antigen unmasking to facilitate labelling for CD3 or feline immunoglobulins was accomplished by incubation with 0.1% trypsin for 30 min at 37°C. Antigen unmasking in sections to be labelled for CD79, BLA36 or MHC Class II was accomplished by exposure to microwaves (7 min, medium power, 650 W microwave oven) after immersion in citrate buffer (10 mM, pH 6.0).

Immunohistochemical labelling for CD3 was carried out by serial incubations at room temperature with normal goat serum (diluted 1 in 5 in phosphate-buffered saline [PBS] 0.01 M, pH 7.4; 30 min), rabbit anti-human CD3 peptide (Dako, Glostrup, Denmark; 1 in 200 in PBS; 4 h), biotin-labelled goat anti-rabbit IgG (Sigma, Poole, Dorset; 1 in 20 in PBS; 30 min) and avidin-peroxidase complex (Sigma; 1 in 20 in PBS; 30 min). In all immunohistochemical procedures, sections were washed twice (15 min) in PBS between each incubation, and antibody labelling was “visualized” by means of diaminobenzidine (Dako) with peroxide, before a light counterstain with Mayer’s haematoxylin.

Immunohistochemical labelling for CD79, BLA36 or MHC Class II necessitated serial incubation with normal goat serum (as above), murine monoclonal anti-human CD79 peptide (Dako; 1 in 100 in PBS; 4 h), murine monoclonal anti-B lymphocyte antigen 36 kD (Dako; 1 in 20 in PBS; 4 h) or murine monoclonal anti-human MHC Class II (Dako; 1 in 20 in PBS; 4 h), followed in each case by biotin-labelled antimouse IgG (Sigma; 1 in 20 in PBS; 30 min) and avidin-peroxidase complex as above.

Feline IgG labelling necessitated serial incubation with normal goat serum (as above), rabbit anti-cat IgG (Nordic Laboratories, Tilburg, The Netherlands; 1 in 50 in PBS; 30 min) and peroxidase-conjugated goat anti-rabbit IgG (Sigma; 1 in 100 in PBS; 30 min). Labelling for IgM or immunoglobulin light chain was carried out by incubation with normal rabbit serum (1 in 5 in PBS, 30 min), goat anti-cat IgM (Nordic Laboratories) or goat anti-human λ light chain or goat anti-human κ light chain (Sigma; all 1 in 50 in PBS; 30 min) and peroxidase-conjugated rabbit anti-goat IgG (Sigma; 1 in 200 in PBS; 30 min).

### Polymerase Chain Reaction (PCR) Analysis of Tumour Tissue for FeLV and FIV Provirus

Five to eight sections, each of 10 μm thickness, were cut from tissue block, dewaxed in xylene, washed in ethanol and digested with proteinase K in DNA lysis solution (10 mM Tris, pH 8.0; 5 mM ethylene-diaminetetraacetic acid [EDTA], pH 8.0; 100 μg proteinase K; 1% sodium-dodecyl-sulphate [SDS]) at 37°C. DNA was purified by extraction with phenol-chloroform followed by precipitation in ethanol, and the DNA pellet obtained was resuspended in 20 μl of triethanolamine buffer, pH 8.0.

The primer sequences used to amplify a 166 bp segment of the FeLV U3 LTR region (Jackson et al., 1993) were: 5′-TTACTGAAGTATGTCCCATG-3′ and 5′-CTGGGGAGCCTGGAGACTGCT-3′. For the amplification of FeLV proviral DNA sequences, reaction mixtures for PCR consisted of 1 × PCR buffer (20 mM (NH₄)₂SO₄; 75 mM Tris-HCl; pH 9.0; 0.1% Tween; 1.5 mM MgCl₂); 200 μM of each of the deoxynucleoside triphosphates (dNTPs); 25 pM of each primer; 500 ng of DNA template, and double-distilled H₂O to a volume of 25 μl. The reaction mixture was overlaid with mineral oil and cycled on a Corbett Thermocycler (Corbett Research, Australia). After initial denaturation of the mixture at 94°C for 3 min, 0.5 unit of Taq DNA polymerase (Perkin Elmer, USA) was added. DNA templates were denatured at 94°C for 1 min, annealed at 52°C for 1 min, and extension was carried out at 72°C for 2 min. This cycle was repeated 35 times. The positive control for FeLV proviral DNA amplification was obtained from formalin-fixed, paraffin wax-embedded tissue.
from a clinical case of lymphoma which was FeLV-positive by enzyme-linked immunosorbent assay (ELISA). The negative control was a formalin-fixed, paraffin wax-embedded tissue sample from a cat from a colony known to be free of FeLV. A negative control with only the PCR reagents, but without DNA template, was also used in each PCR run to ensure that no contamination was present.

For amplification of FIV proviral DNA, the reaction mixture was similar to that used for FeLV, with the substitution of 10 pM of the FIV-specific primers (Greene et al., 1993; Kyaw-Tanner et al., 1994) in place of the FeLV primers. Two oligonucleotide primer pairs were used to amplify regions of env and gag within the genome of FIV. The first set of primer sequences was used to amplify a 568 bp segment of the FIV env gene (gp120) and have the sequence: 5' AGGACCAGAAAGGCTGAAGA 3' and 5' TTCTGGTGCCAACAAATCCCA 3' (Kyaw-Tanner et al., 1994). The second set of primer sequences was used to amplify a 467 bp segment of the FIV gag gene and have the sequence: 5' CTAAGCTGCTGAGCTGA 3' and 5' ACAACTGCAACCTAGCTGGTGCAAA 3' (Greene et al., 1993). For both sets of primers, the PCR conditions consisted of an initial denaturation of 5 min at 94°C, followed by 30 cycles each of (1) denaturation at 94°C for 30 sec, (2) primer annealing at 60°C for 1 min, and (3) primer extension at 72°C for 2 min. Genomic DNA extracted from an FIV-infected cat from which virus had been repeatedly isolated was used as a positive control. The negative control was DNA from a cat known to be free of FIV. After amplification, PCR products of FeLV and FIV proviral DNA were electrophoresed first through 4% NuSieve agarose (FMC BioProducts, USA) and then through 1.5% agarose (Promega Corporation, USA), and stained with ethidium bromide 0.5 µl/ml.

For both FIV and FeLV, the specificity of PCR-amplified proviral DNA products was confirmed by Southern blot analysis. Digoxigenin (DIG) (Boehringer Mannheim, Australia)-labelled probes were prepared by PCR. The sequences of the probes were initially confirmed by purifying the plasmid DNA over QIAGEN columns (QIAGEN Pty Ltd, Australia) and sequencing the purified DNA with M13 universal sequencing primers (Promega Corporation, USA) and ABI Prism™ Dye Cycle Sequencing kit (Applied Biosystems, Foster City, USA). After electrophoresis of PCR products, gels were transferred to hybond-N (Amersham, England) filters, which were then UV-cross linked. After prehybridization for 2 h in 5 x saline sodium citrate (SSC), containing DIG-blocking solution 1%, N-lauroylsarcosine 0.1% and SDS 0.2%, the membranes were hybridized in the same buffer containing freshly denatured PCR-labelled probe at 68°C overnight. Filters were washed twice in 2 x SSC containing SDS 0.1% at room temperature, followed by high stringency washing in 0.1% x SSC containing SDS 0.1% at 68°C. Samples containing DNA obtained from both FIV-negative as well as FeLV-negative cats were also assayed, and in no case did DIG-labelled probes react with such controls.

**Results**

**Clinical Features**

The clinical features of the eight cases are summarized in Table 1. The cats ranged in age from 4.5–18 years (mean 11.4±3.9 years; n=7) and all were domestic shorthair animals. There were four females (three neutered) and four males (three neutered). On initial examination, all cases showed either one (n=7) or two (n=1) palpable masses in the submandibular or ventral cervical region, and in one cat (case 4) there was also enlargement of one prescapular lymph node. In three cases the masses were reported as rapidly growing, but in one further cat the tumour grew slowly. Two cats (cases 4 and 6) tested for FeLV/FIV at the time of presentation were negative for
T-cell-rich B-cell Lymphoma in the Cat

Table 1
Clinical features of the study population

<table>
<thead>
<tr>
<th>Case</th>
<th>Age (years)</th>
<th>Breed</th>
<th>Sex</th>
<th>FeLV/FIV status</th>
<th>Clinical presentation</th>
<th>Follow-up information</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15</td>
<td>DSH</td>
<td>F</td>
<td>NT</td>
<td>Mass, 5 cm long, in ventral neck</td>
<td>Intermittent vomiting, but no recurrence at 6 months</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>DSH</td>
<td>FN</td>
<td>NT</td>
<td>Rapidly growing mass in ventral neck, 4 x 2.5 x 1.5 cm</td>
<td>No recurrence and clinically normal at 6 months</td>
</tr>
<tr>
<td>3</td>
<td>11</td>
<td>DSH</td>
<td>FN</td>
<td>NT</td>
<td>Rapidly growing mass, left ventral neck, 2 x 2 x 1.5 cm</td>
<td>NA</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>DSH</td>
<td>MN</td>
<td>Negative</td>
<td>Mass removed from ventral neck; enlarged prescapular lymph node also removed</td>
<td>Euthanasia after diagnosis</td>
</tr>
<tr>
<td>5</td>
<td>NR</td>
<td>DSH</td>
<td>M</td>
<td>NA</td>
<td>Two slow growing masses, right submandibular region</td>
<td>NA</td>
</tr>
<tr>
<td>6</td>
<td>4.5</td>
<td>DSH</td>
<td>MN</td>
<td>Negative on two occasions</td>
<td>Single mass, left submandibular region</td>
<td>Recurrence at 6 and 12 months; chemotherapy and no recurrence at 18 months</td>
</tr>
<tr>
<td>7</td>
<td>11</td>
<td>DSH</td>
<td>FN</td>
<td>NA</td>
<td>Anterior submandibular mass, 3 x 2 x 1 cm</td>
<td>NA</td>
</tr>
<tr>
<td>8</td>
<td>18</td>
<td>DSH</td>
<td>MN</td>
<td>NT</td>
<td>Rapidly growing mass, right submandibular region</td>
<td>No recurrence at 6 months</td>
</tr>
</tbody>
</table>

NR, not recorded; NT, not tested; NA, information not available; DSH, domestic shorthair; F, female; FN, neutered female; M, male; MN, neutered male.

both viruses. In each of the eight cases, the mass was completely excised and submitted for histopathological examination.

Follow-up information was obtained in five cases. One of these cats was humanely killed following histopathological diagnosis, and three cats had no recurrence of the tumour 6 months after resection. In one cat (case 6), the original tumour was removed from the left submandibular region; a second mass was removed from the ventral cervical area 6 months later, and a third tumour from the left submandibular region was removed 12 months after initial presentation. At this time the cat was retested for FeLV and FIV with negative results and a course of chemotherapy with cyclophosphamide, vincristine and prednisolone was initiated. At the time of writing (18 months after initial diagnosis) there has been no further recurrence of the tumour.
Histopathological Features

In each case, normal lymph node architecture was virtually obliterated by a diffuse ($n=4$) or nodular ($n=4$) proliferation of lymphoid cells (Fig. 1). This was a heterogeneous population of well-differentiated small lymphocytes, lymphoblasts and histiocytic lymphocytes. Scattered throughout were very large (up to 100 μm) cells, each with a large, round to oval nucleus and a variable quantity of eosinophilic cytoplasm. The nuclear outline was often irregular or cleaved and occasional binucleate or multinucleate forms were present. The nuclei of these cells were vesicular, with scant chromatin forming peripheral aggregates. Nucleoli were sometimes inapparent, but generally a single central nucleolus was observed. This cytological appearance is consistent with that of the “popcorn” cells described in certain human lymphoid tumours (Fig. 2). The mitotic rate of each tumour was low (Table 2), but the lesions were characterized by the presence of bizarre mitoses that were generally associated with the giant cell population.

Immunohistochemical Features

These are summarized in Table 2. In each case, the dominant population of small to blastic lymphocytes comprised a mixture of $\text{CD}3^+$ T lymphocytes (Fig. 3) and $\text{BLA36}^+$ B lymphocytes (Fig. 4). T and B cells were generally present in approximately equal proportion; in cases 2 and 3, however, B cells predominated, and in case 8 T cells did so. Relatively few of the $\text{BLA36}^+$ B
T-cell-rich B-cell Lymphoma in the Cat

Fig. 2. Section of lymph node from case 2, showing a mixed population of small to blastic lymphocytes, with prominent “popcorn” cells with giant, vesicular nuclei. HE. × 125.

<table>
<thead>
<tr>
<th>Case</th>
<th>Mitotic rate*</th>
<th>Large cells</th>
<th>Small lymphocytes†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CD3 CD79 BLA36 MHC II</td>
<td>CD3 CD7 BLA36 MHC II</td>
<td>CD3 CD7 BLA36 MHC II</td>
</tr>
<tr>
<td>1</td>
<td>1.5 ± 1.28</td>
<td>– – + – –</td>
<td>50% – 50% 95%</td>
</tr>
<tr>
<td>2</td>
<td>0.8 ± 0.98</td>
<td>– – + + – –</td>
<td>30% 50% 70% 75%</td>
</tr>
<tr>
<td>3</td>
<td>1.5 ± 0.64</td>
<td>– – + – –</td>
<td>30% 10% 70% 95%</td>
</tr>
<tr>
<td>4</td>
<td>1.9 ± 1.15</td>
<td>– – + + –</td>
<td>50% 10% 50% 95%</td>
</tr>
<tr>
<td>5</td>
<td>0.9 ± 1.04</td>
<td>– – + – –</td>
<td>50% 5% 50% 95%</td>
</tr>
<tr>
<td>6</td>
<td>2.0 ± 1.18</td>
<td>– – + + –</td>
<td>50% 50% 50% 95%</td>
</tr>
<tr>
<td>7</td>
<td>0.0 ± 0.0</td>
<td>– – + + –</td>
<td>50% – 50% 75%</td>
</tr>
<tr>
<td>8</td>
<td>2.2 ± 1.6</td>
<td>– – + + –</td>
<td>70% – 30% 75%</td>
</tr>
</tbody>
</table>

*Mitotic rate: mean ± standard deviation of mitoses counted in 10 high-power (× 40 objective) fields.
† For small lymphocytes, the percentages refer to the positively stained lymphocytes as determined by semi-quantitative assessment of 10 high-power (× 40 objective) fields.
Ig, includes immunolabelling for γ, µ, λ, and κ immunoglobulin chains.

cells also expressed CD79, except in cases 2 and 6, in which most B cells had dual expression of these markers. The majority (75 to ≥95%) of the T and B cells expressed MHC Class II. The bizarre giant cells were uniformly negative for expression of CD3 and CD79, in addition to the immunoglobulin heavy and light chain markers. However, in all cases these cells showed strong membrane expression of BLA36 (Fig. 5), and in five cases most of the giant cells showed distinct membrane expression of MHC Class II (Fig. 6).
Fig. 3. Section of lymph node from case 5 treated to show the pan T lymphocyte marker CD3. There is clear membrane labelling of the majority of smaller lymphocytes, but “popcorn” cells remain unlabelled. A mitotic figure is present in one such cell (arrow). Avidin-biotin immunoperoxidase. Anti-CD3. × 150.

Fig. 4. Section of lymph node from case 2 treated to show the B lymphocyte marker BLA36. There is clear membrane labelling of many of the smaller lymphocytes, in addition to the large “popcorn” cells. Avidin-biotin immunoperoxidase. Anti-BLA36. × 150.
Fig. 5. Section of lymph node from case 7 treated to show the B lymphocyte marker BLA36. The majority of smaller lymphocytes are unlabelled, but there is strong membrane labelling of the large “popcorn” cells. Avidin-biotin immunoperoxidase. Anti-BLA36. ×125.

Fig. 6. Section of lymph node from case 7 treated to show MHC Class II molecules. There is membrane labelling of many of the smaller lymphocytes in addition to the large “popcorn” cell in the centre field. Avidin-biotin immunoperoxidase. Anti-MHC Class II. ×125.
Detection of FeLV and FIV Provirus

PCR analysis of tissue from each tumour gave negative results for FeLV and FIV provirus. By contrast, strong signals were obtained with positive control samples.

Discussion

This study reports the features of an unusual form of feline lymphoma which, clinically and pathologically, resembles human “nodular lymphocyte predominance L&H (lymphocytic and histiocytic) Hodgkin’s disease” (NLPHD). The latter falls within the category of T-cell-rich B-cell lymphoma (Warnke et al., 1995). NLPHD occurs most commonly in 30–40 year-old males, and the typical clinical presentation is of an isolated, chronic lymphadenopathy of 0.25–10 years’ duration, most commonly affecting the cervical, axillary or inguinal lymph nodes. After treatment, single or multiple relapses often occur at the original site of the tumour. These features are similar to those of the cats in the present series. There was no clear gender predisposition, but the cats (all domestic shorthair) were generally middle aged to older animals. The clinical presentation was most commonly of enlargement of a single submandibular or cervical lymph node, and local recurrence of the tumour was recorded.

There was also histological similarity between the lesions of human NLPHD and this feline tumour. The microscopical features of NLPHD include the presence of characteristic “L&H” cells within a background of nodular proliferation of small lymphocytes and fewer histiocytes. Some cases lack nodularity and have been termed “diffuse lymphocyte predominance (L&H) Hodgkin’s disease”. The L&H cells are very large, with large vesicular nuclei and usually sparse cytoplasm. The nuclei may be multilobulated or there may be multiple nuclei. These cells are termed “popcorn” cells because of this appearance (Warnke et al., 1995). This microscopical appearance was shown by each of the feline tumours in this study. Both nodular and diffuse forms of the tumour were observed, and although the mitotic rate of the L&H cells was low, the mitoses were generally of an irregular form.

The immunological phenotype of the cells comprising human NLPHD is well-characterized. The L&H cells are of the B lymphocyte lineage, as shown by the expression of a range of surface molecules including CD19, CD20 (pan B cell markers), CD22 (mature B cell marker), CD74 (B cell, monocyte and macrophage marker), Cdw75 (mature B cells and T cell subset) and CD45RA (isoform of the leucocyte common antigen) (Warnke et al., 1995). The cells do not clearly express immunoglobulin heavy or light chains. In some studies, light chain expression has been demonstrated (Schmid et al., 1991), other workers, however, have failed to detect light chain mRNA expression by L&H cells (Momose et al., 1992). The small lymphocytes within the background of the lesions are considered reactive rather than neoplastic, and are of mixed phenotype, including B cells and T cells, particularly of the CD4 type (Warnke et al., 1995).
T-cell-rich B-cell Lymphoma in the Cat

Similar findings are reported for the feline tumours in the present study. The background lymphocytes in each case were a mixture of T and B lymphocytes, the majority of which expressed MHC Class II, as expected for the cat (Hunt et al., 1995). The B lymphocyte component was characterized by expression of the membrane glycoprotein BLA36 and in only two cases did these cells also express CD79, a part of the B-cell immunoglobulin receptor complex. In man, BLA36 is expressed by early and activated B lymphocytes, but not resting or peripheral B cells. It is also present on the membrane of Reed-Sternberg cells and Hodgkin’s cells (Della Croce et al., 1991). In normal feline lymph node, BLA36 expression was largely restricted to germinal centre lymphoblasts, with only scattered positively labelled cells in the follicular mantle zone or paracortex (data not shown). The high proportion of BLA36+ B cells within the lesions of this study, coupled with the lack of expression of CD79, would suggest that this was an irregular population of B lymphocytes, which may have been neoplastic rather than reactive in nature.

The phenotype of the feline L&H cells was also consistent with those of human NLPHD. There was no evidence of expression of CD3, immunoglobulin heavy or light chain, or CD79, but the cells uniformly expressed membrane BLA36, and in five of eight cases surface MHC Class II was also identified. These findings are similar to those in the only reported case of feline T-cell-rich B-cell lymphoma, in which the L&H cells were BLA36+ and occasionally expressed λ light chain (Steele et al., 1997).

Two cats in this study were tested for both FeLV and FIV at the time of clinical diagnosis, with negative results, and PCR analysis did not reveal FeLV or FIV provirus associated with any of the tumours. The results thus provide definite evidence that this form of feline lymphoid neoplasia is not FeLV-associated; this conclusion is consistent with the relatively long clinical course and favourable prognosis, neither of which is characteristic of most FeLV-induced lymphomas in the cat. The possible role of FIV is less certain, as FIV provirus has been detected in some lymphomas by PCR (Endo et al., 1997), but not in other FIV-positive cats when tumours were examined by Southern blotting (Callanan et al., 1996). The absence of FIV provirus by PCR in all cases in the present study, and the fact that two cats were serologically negative for FIV antibody at the time of presentation, strongly suggests that FIV plays no aetiological role in this form of lymphoma.

In summary, this report describes eight cases of a unique form of feline lymphoma, with clinical, histological and immunohistochemical features consistent with human NLPHD. These tumours were recognized within a 3-year period, and retrospective study (to 1990) of archival material at the University of Bristol failed to identify further cases. These findings may indicate the emergence of a new feline disease.

Acknowledgments

The authors gratefully acknowledge the contribution of those veterinary surgeons who submitted material and responded to requests for follow-up information. We thank Dr Joya Pawade, Department of Histopathology, Bristol Royal Infirmary, for helpful discussion.
References


T-cell-rich B-cell Lymphoma in the Cat


[Received, August 3rd, 1998]
[Accepted, September 30th, 1998]