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TECHNICAL REPORT

Feline low-grade intestinal T cell lymphoma: a unique natural model of human indolent T cell lymphoproliferative disorder of the gastrointestinal tract

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Abstract

Indolent T cell lymphoproliferative disorder (LPD) of the gastrointestinal tract (GI-TLPD) is a rare human primary gastrointestinal T cell lymphoma that was recently included in the 2016 revision of the World Health Organization classification of lymphoid neoplasms. Low-grade intestinal T cell lymphoma (LGITL), an emerging disease in the domestic cat, shares a number of features with human GI-TLPD. In this prospective study, we determined whether feline LGITL might serve as a model of human GI-TLPD. We analyzed clinical, laboratory, and radiological data and performed histopathological and molecular studies on small intestinal biopsies from 22 domestic cats diagnosed with LGITL. This cancer mostly affects aging cats, is associated with nonspecific gastrointestinal tract signs, and is usually characterized by an indolent course. A histopathological analysis indicated that LGITL was mainly located in the jejunum. The small intestinal lamina propria was infiltrated by large numbers of small CD3+ T cell lymphocytes with various CD4 and CD8 expression profiles (CD4+ CD8− (4 out of 11, 36%), CD4− CD8+ (3 out of 11, 27%), and CD4− CD8− (4 out of 11, 36%)). Intraepithelial lymphocyte (IEL) counts were elevated in all cases. Ki67 was expressed in lamina propria lymphocytes and IELs at a low level (<30%). Most LGITLs were labelled by antibodies against phosphorylated STAT5, but were negative for CD56 and phosphorylated STAT3. T cell receptor gamma chain gene monoclonality was found in 86% of cases. These findings confirmed that feline LGITL shares clinical and histopathological features with human GI-TLPD. Feline LGITL may therefore constitute a relevant model of the human disease.

Introduction

Primary lymphoma of the gastrointestinal (GI) tract is the most frequent type of primary extranodal non-Hodgkin

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lymphoma (NHL). More than 85% of cases are of B cell origin. In contrast, T cell primary gastrointestinal lymphoma is a rare entity; it accounts for <5% of NHLs [1]. An entity provisionally named indolent T cell lymphoproliferative disorder of the GI tract (GI-TLPD) has been added to the 2016 revision of the World Health Organization (WHO) classification of primary intestinal T and natural killer (NK) cell lymphomas [2]. GI-TLPD was first described by Carbonnel et al. in 1994 [3] and has a relatively indolent course. Although precise diagnostic criteria have not yet been finalized, the case reports and small case series published over the past 20 years have given some insights into the disease's clinical presentation, histopathology, immunohistochemistry, and clonality [1, 4–6]. However, this rare disease is often misdiagnosed, and there is currently no consensus on the most effective, safest treatment options. Consequently, the majority of patients with GI-TLPD still show persistent clinical signs and poor quality of life [5, 6].

Over the past two decades, the veterinary community has described an emerging GI lymphoma subtype (feline low-grade intestinal T cell lymphoma, LGITL) in the aging domestic cat. In fact, LGITL is the most frequent gastrointestinal tract cancer in this species [7]. LGITL was first compared to monomorphic epitheliotropic T cell lymphoma (MEITL), in view of the associated infiltration of the lamina propria and epithelium by medium-sized monomorphic neoplastic T cells [8]. However, the clinical, laboratory, histological, and immunohistochemistry features of LGITL are very similar to those of GI-TLPD. As a result, LGITL was cited as a relevant model of human GI-TLPD in two recent publications [7, 9]. Little is known about the mechanisms underlying the pathogenesis of indolent TLPDs in either species. The objective of the present prospective study was to extensively define feline LGITL on the clinical, laboratory, radiological, histologic, and molecular levels, and thus assess its relevance as a model of human GI-TLPD.

Methods

Recruitment of cases of LGITL

The study was approved by the investigational review board at Alfort National Veterinary School (COMERC, Maisons-Alfort, France; reference: 2017-05-09). Between July 2016 and July 2018, gastrointestinal tract biopsies were collected from all cats referred to Alfort National Veterinary School's small animal clinic for suspected LGITL. Tissue sections were reviewed by board-certified veterinary and human pathologists (NC and JB, respectively); both were blinded to the subject's clinical history. Given that the human and veterinarian classification systems overlap extensively, biopsies were classified according to the 2016 revision of the WHO classification of human lymphoma [2], the WHO classification of canine lymphoma [10], and the sixth edition of Jubb, Kennedy, and Palmer's "Pathology of Domestic Animals." Ultimately, 22 cats with a final histological diagnosis of LGITL were included.

Histopathology

The following criteria were specifically assessed: the topography of the cellular infiltrate (epithelium, lamina propria, submucosa, and muscularis propria or serosa), cytological features, cellularity gradient, villous atrophy (i.e., a villus-to-crypt ratio below 3:1) [11], crypt hyperplasia, epithelial apoptosis in the crypt (defined as a cumulative total of more than four apoptotic bodies in ten consecutive crypts) [12], lymphocytic cryptitis (i.e., at least 30 intraepithelial lymphocytes (IELs) per 100 enterocytes in the crypts), crypt abscesses (i.e., an accumulation of neutrophils within the crypt lumens), and lamina propria fibrosis. Patchy lesions were defined as inhomogeneously distributed small neoplastic lymphocytes within the epithelium or the lamina propria. Masson's trichrome staining was used to reveal the extent of fibrosis, if present.

Immunohistochemistry

The expression of each cluster of differentiation (CD) on formalin-fixed, paraffin-embedded tissue sections was visually and independently assessed by the two pathologists. Sections from each sample were labelled with a panel of antibodies against CD3 (1:20 dilution, F7.2.38, DakoCytomation, for the identification of T cell lineages), CD20 (1:400 dilution, RB-9013-P1c, Lab Vision Corporation, for the identification of B cell lineages), myeloid/histiocyte antigen (1:200 dilution, MAC387, DakoCytomation, for macrophages), CD56 (ready-to-use primary antibody, CD564, Leica), phosphorylated human STAT3 (pSTAT3)^{Y705} (1:100 dilution, D3A7, Cell Signaling) and pSTAT5$^{Y694/699}$ (1:100 dilution, polyclonal antibody, Biorbyt, for STAT3/STAT5 pathway activation), and Ki67 (1:75 dilution, MIB-1, DakoCytomation, for assessment of the proliferative index). CD4 and CD8 expression was evaluated on frozen biopsies from 11 cats using antibodies against CD4 (1:200 dilution, 3-4F4, Southern Biotech) and CD8 (1:200 dilution, fCD8 (FT2), Southern Biotech). If the infiltration was not evenly distributed, the most infiltrated areas were examined. Lastly, we performed a semiquantitative immunohistochemical assessment of the epithelium and the lamina propria (Supplemental Fig. 1). Discordant cases were resolved in a consensus review with a multihead microscope.

Clonality analysis

Clonal T cell receptor gamma (*TCRγ*) chain gene rearrangement was detected in DNA extracted from a tumor biopsy ($n = 22$) and a matched peripheral blood sample ($n = 14$) using a multiplex polymerase chain reaction assay (Genefast Laboratori, Forlì, Italy), as previously reported [13]. The results were reviewed by specialists with expertise in human or veterinary clonality assays. A rearrangement was considered to be monoclonal when only one peak was detected and polyclonal when no peaks or more than 4 peaks were detected.

Statistical analysis

Overall survival was defined as the time interval between the first day of treatment and death (from any cause) or last follow-up. The survival curve was plotted according to the Kaplan–Meier method.

Results

Epidemiology

Eighteen out of the 22 cats were domestic shorthairs (82%). There were 16 males (73%) and 6 females (27%), and all had been spayed or castrated. Ten of the 22 cats (45%) were kept indoors exclusively. The median age and weight at presentation were respectively 13 years [range 8–16] and 4.0 kg [range 2.2–7.4]. All were fed with regular, commercially available cat food. The epidemiological data are detailed in Supplemental Table 1.

Clinical features

The median time interval between the onset of signs and presentation was 365 days [range 62–1460]. The main clinical signs included weight loss ($n = 17$, 77%), vomiting ($n = 15$, 68%), and small intestinal diarrhea ($n = 14$, 64%). Abnormal findings upon abdominal palpation were noted for 16 cats (73%)—mostly thickening of the intestinal loops in the absence of an identifiable mass. The clinical data are detailed in Supplemental Table 2.

Laboratory data

Elevated feline-specific pancreatic lipase (>5.3 µg/L, suggesting concomitant pancreatitis) was observed in 6 of the 19 cases with data (32%). One cat displayed hyperthyroidism. A modification of the liver enzyme profile was rare ($n = 3$, 14%). Mild hypoalbuminemia was recorded in three cases (14%), while an elevated creatinine level (≥18 mg/L) was observed in 4 of the 22 cases (18%). A low serum cobalamin level (<200 pg/ml) was detected in 12 of the 21 cats (57%). Anemia was observed in only 4 of the 22 cats (18%). Six of the 22 cats (27%) had mild-to-moderate leukocytosis (mostly due to neutrophilia), while 8 cats (36%) showed moderate lymphopenia.

The laboratory data are detailed in Supplemental Tables 3 and 4.

Ultrasound findings

A thickened muscularis propria layer was observed in all cases. Sixteen cats (73%) had thickened mesenteric lymph nodes (>5 mm). Abdominal effusion was noted in 10 of the 22 cases (45%).

Treatments and outcomes

As described previously [14] and in view of the problem of treatment compliance in cats, combination chemotherapy with high-dose pulse chlorambucil (15 mg/m^2 orally once a day (SID), every 3 weeks for 4 consecutive days) and prednisolone (3 mg/kg given orally SID, with the dose tapered to 1 mg/kg orally once clinical remission was achieved) was implemented. If no adverse events were observed, the regimen was continued indefinitely.

The median overall survival time was 329 days (Supplemental Fig. 2). Twelve of the 22 cats (55%) died during the study. Given that LGITL affects elderly cats with comorbidities, 8 of the 12 deaths were not related to LGITL; the causes were variously other cancers ($n = 3$), postoperative complications ($n = 2$), pulmonary embolism ($n = 1$), feline infectious peritonitis ($n = 1$), and hypovolemic shock ($n = 1$). Two cats died from a gastrointestinal tumor (suspected clinically to be a high-grade lymphoma), respectively, 96 and 390 days after the diagnosis of LGITL. Unfortunately, a histopathological examination could not be performed and so no evidence of LGITL transformation was obtained. Lastly, two cats were euthanized and no information on their clinical condition at the date of death was available.

Many owners refused follow-up consultations, and so the median length of follow-up for living cats was only 87 days. However, two cats with LGITL are still being followed up in the clinic; they showed persistent clinical remission, respectively, 694 and 724 days after the diagnosis of LGITL.

Tumor site and extent of the disease

Eighteen of the 22 cats (82%) were diagnosed with LGITL in the jejunum, 6 of the 22 cats (27%) had infiltration in both jejunum and ileum, and 2 cats (9%) displayed infiltration in the ileum only. In agreement with the literature data [7, 8],

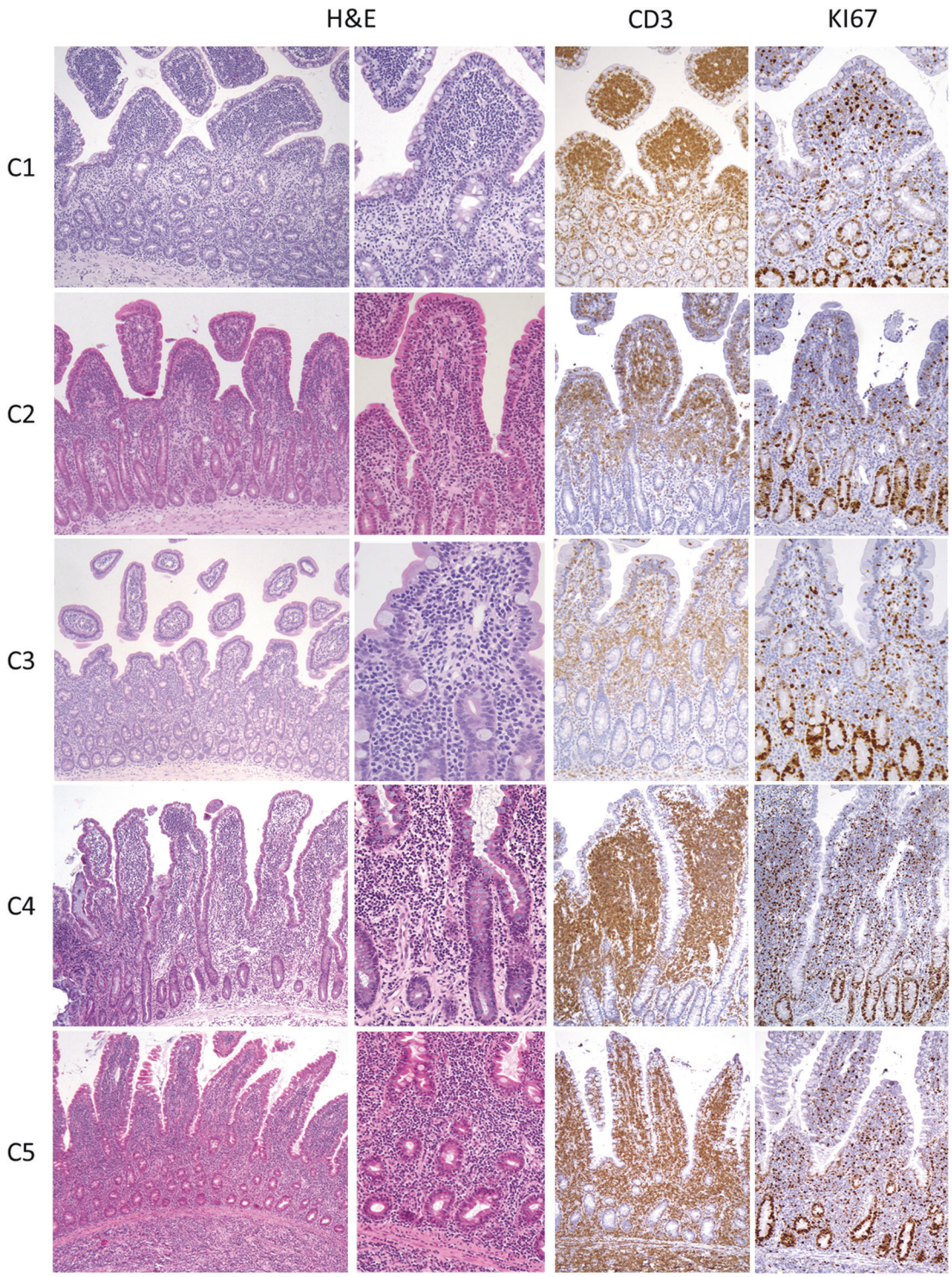

Fig. 1 Hematoxylin and eosin (H&E), CD3, and Ki67 immunohistochemical staining in feline LGITL. C1 to C5 correspond to five distinct individuals diagnosed with LGITL. C1, C2, and notably C3 show a variable apical-to-basal gradient, while the infiltration is more diffuse in C4 and C5. The degree of villous atrophy is variable: the villi are shorter and larger in C1, C2, and C3, whereas no atrophy is observed in C4 and C5. Ki67 labelling is of mild-to-moderate intensity in all LGITL cases (10–30% of labelled cells). The magnification of the original image was ×40 and 100 for H&E staining, and ×100 for CD3 and Ki67 immunolabelling.

large intestine biopsies were not performed because neither the clinical signs nor the ultrasound findings suggested colonic infiltration. Duodenal infiltration was identified on one endoscopic biopsy, but the cat's owner refused exploratory laparotomy. Jejunal and/or ileocolic lymph node biopsies were performed for all but three of the cats.

Histological findings

The most common histopathologic feature was massive infiltration of the lamina propria and the epithelium by small monomorphic lymphoid cells. In 5 cats, the epithelial infiltration was characterized by clusters of IELs. Patchy lesions in the lamina propria and the epithelium were observed in 5 and 11 of the 22 cases, respectively.

An apical-to-basal gradient was observed in 9 of the 22 cases (41%) (Fig. 1). Intestinal villous atrophy (Fig. 1) and mild-to-severe lymphocytic cryptitis were frequent (19 out of 22 (86%) and 20 out of 22 (91%) cases, respectively). The depth of infiltration into the intestinal wall was variable: submucosal, muscularis, and serosal (Fig. 1) infiltrations by neoplastic lymphocytes were observed in 68%, 48%, and 24% of the cases, respectively. Interestingly, Masson's trichrome staining revealed fibrosis only in the deep part of the lamina propria and the submucosa in 9 of the 21 (43%) documented cases of LGITL. A histopathological assessment of the lymph nodes showed follicular lymphoid hyperplasia with no obvious signs of tumor infiltration. The pathology findings are detailed in Table 1.

Table 1 Pathologic findings for 22 cats with LGITL.

Histological criteria	Number of cases (%)
Villi	
Villous atrophy	19/22 (86%)
Epithelium	
Epithelial infiltration by small lymphocytes	19/22 (86%)
Patchy infiltration by small lymphocytes	11/22 (50%)
Clusters of intraepithelial lymphocytes	5/22 (23%)
Lamina propria	
Dense infiltration of lamina propria	21/22 (95%)
Patchy infiltration of lamina propria	5/22 (23%)
Apical-to-basal gradient of small lymphocytes	9/22 (41%)
Crypts	
Compensatory hyperplasia	17/22 (77%)
Lymphocytic cryptitis	20/22 (91%)
Neutrophilic cryptitis	3/22 (14%)
Abscesses	6/22 (27%)
Epithelial apoptosis	1/22 (5%)
Morphology	
Monomorphic small lymphoid cell population in the lamina propria and epithelium	22/22 (100%)
Monomorphic small lymphoid cell population mixed with an inflammatory population	10/22 (45%)
Depth of infiltration	
Submucosa infiltration by the lymphocytic population	15/22 (68%)
Muscularis infiltration by the lymphocytic population	10/21 (48%)[a]
Serosal infiltration by the lymphocytic population	5/21 (24%)[a]
Fibrosis	
Fibrosis located in the lamina propria	16/21 (76%)[a]
Fibrosis exclusively located in the deep lamina propria and the submucosa	9/21 (43%)[a]

[a]Deep infiltration was assessed in all the cats having undergone a surgical biopsy ($n = 21$).

Immunohistochemical assessments

The whole lamina propria of the small intestine was massively infiltrated by small T CD3+ lymphocytes (Fig. 1). Among the 11 cases analyzed, the CD4 and CD8 expression profiles were variable (CD4+ CD8−: $n = 4$, 36%; CD4− CD8+: $n = 3$, 27%; CD4− CD8−: $n = 4$, 36%; Fig. 2A). A significant elevation of the CD3+ T cell count in the epithelium was also observed (median count: 97 per 100 cells [range 20–100]).

As expected, the proportions of CD20+ B cells in the epithelium and the lamina propria were very low (5% [range 1–5] and 20% [range 10–40], respectively). Elevated values were still typically observed in the lamina propria for cases with an apical-to-basal gradient of neoplastic cell infiltration. The median Ki67 proliferation index was 20% [range 5–45] in the epithelium and 30% [range 7.5–65] in the lamina propria (Fig. 1). Staining for CD56 was negative in 19 of the 20 cases (95%). Only one case had CD56+ lymphocytes in the lamina propria but not the epithelium (Fig. 2B). Staining for pSTAT3 was negative in all cases, and staining for pSTAT5 was positive in all cases (Fig. 3). The main immunophenotypic findings are listed in Table 2.

Clonality results

At the time of diagnosis, the molecular detection of *TCRγ* rearrangements revealed monoclonality in 19 of the 22 cases (86%). The various clonality profiles are shown in Supplemental Fig. 3. A circulating clone with the same clonal rearrangement was noted in the only case to present with lymphocytosis at diagnosis.

Discussion

Researchers have long used animal models to better characterize human diseases. The mouse is the most frequently used animal model; it has several advantages (such as a short gestation time and low cost), but is primarily limited by the need to induce tumors. Furthermore, mice models do not reproduce genomic instability and heterogeneity in tumor cells and the tumor microenvironment [15]. In contrast, naturally occurring tumors in dogs (sarcomas,

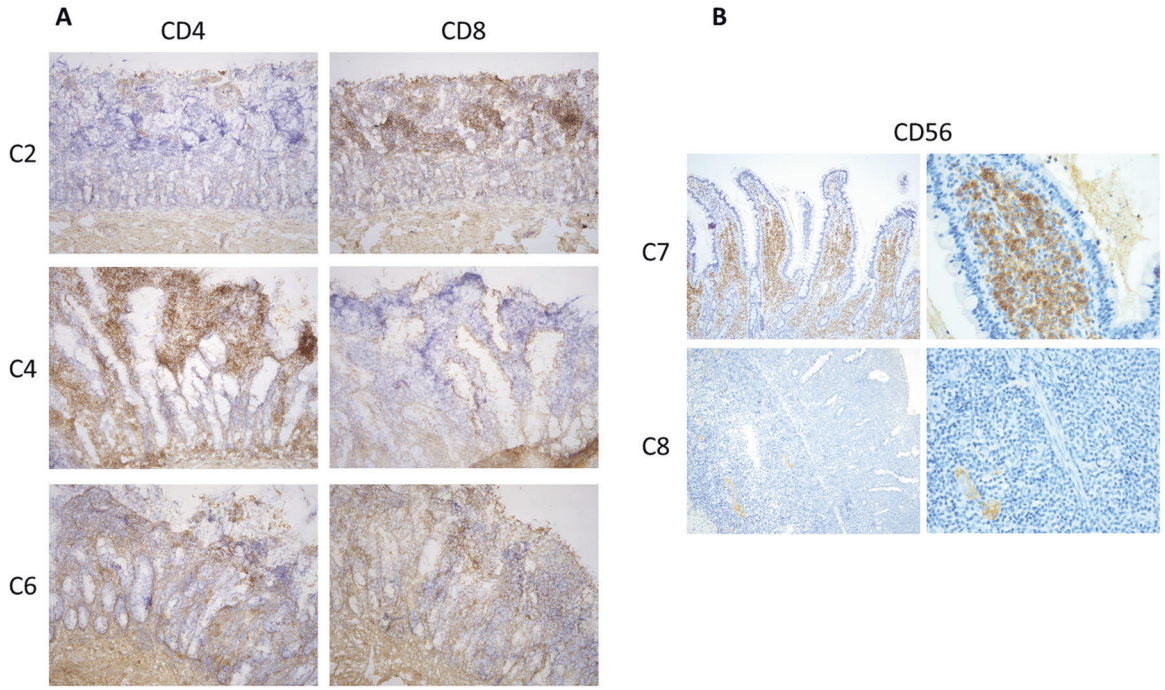

Fig. 2 CD4, CD8, and CD56 immunohistochemical staining in feline LGITL. A Different profiles were observed. C2: CD8+; C4: CD4+; C6: CD4−/CD8− (magnification of the original image: ×40). **B** C7 is the only CD56+ case in the series. C8 stained negative, and nerve fibers in the lamina propria served as an internal positive control (magnification of the original image: ×40 and ×200).

osteosarcomas, or lymphomas) are well-established models for several human cancers and share many characteristics—including the histological appearance, tumor genetics, biological behavior, and response to conventional treatments [16, 17]. The greater emphasis to date on dogs is probably also due to the fact that the canine genome was sequenced in 2005 [18], whereas the complete feline sequence was published only recently [19]. However, comparative oncology with feline models is now developing because the cat may be a good model of oral squamous carcinoma, thyroid carcinoma, and mammary carcinoma; these tumors are more frequent in cats than in humans, and the response to treatment is more predictable in cats than in laboratory animals [20].

Accordingly, the objective of the present prospective case series was to better characterize feline LGITL, which has already been suggested as a potential model of human GI-TLPD in two recent reports [7, 9]. We compared the clinical, laboratory, histopathological, and molecular findings from 22 cases of feline LGITL with the literature data on the main subtypes of human primary intestinal T cell lymphoma, that is, GI-TLPD, enteropathy-associated T cell lymphoma (EATL), and MEITL. Although feline LGITL was initially reported to be similar to MEITL (previously referred to as type II EATL) [8], the hallmarks of MEITL (i.e., medium-sized tumor cells, CD56 positivity, and constant epitheliotropism [2]) are not in fact observed in feline LGITL. Our results showed that feline LGITL shares a large number of features with GI-TLPD (Table 3), but also highlighted some cat-specific features. While the course of the disease is indolent in both species, most humans with GI-TLPD have persistent symptoms; this contrasts with the lasting remission described for cases of feline LGITL treated with chlorambucil and prednisolone. With regard to histopathological features, epithelial lymphocytosis, villous atrophy, cryptitis, or gradient in the density of neoplastic cells are observed often in feline LGITL but rarely in human GI-TLPD [1, 21]. In humans, elevated epitheliotropism and villous atrophy are the two main distinguishing features vs. celiac disease. However, several aspects ruled out this diagnosis in our feline cases. First, the lamina propria in celiac disease contains a mixture of plasma cells, lymphocytes, occasional eosinophils, and macrophages, but is never infiltrated by monomorphic small T cells [22]. Second, the absence of gluten in regular cat food means that celiac disease (an autoimmune disorder leading to gluten intolerance) has not been reported per se in cats.

Feline LGITL is expected to exhibit oligoclonal or monoclonal TCR rearrangements. However, we detected only three polyclonal cases. Unexpectedly, one of these cases exhibited all the clinical and histopathological features of LGITL, with deep, massive, monomorphic T cell infiltration—suggesting a possible technical issue in the TCRγ rearrangement assay. The other two cases displayed a polymorphic infiltrate with a low proportion of tumor cells, which might explain why clones were not detected.

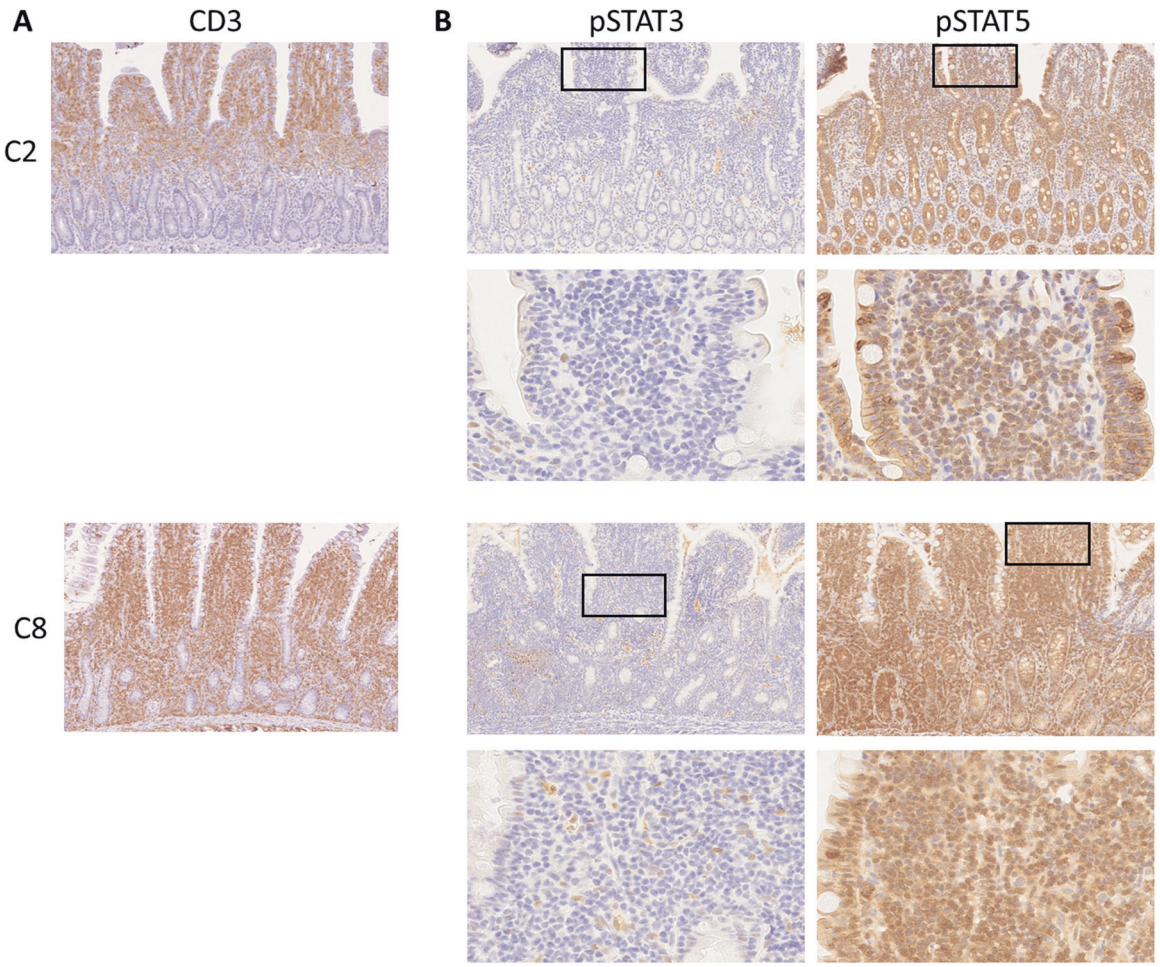

Fig. 3 CD3, pSTAT3, and pSTAT5 immunohistochemical staining in two representative cases of feline LGITL (C2 and C8). A The tumor cells stained positive for CD3 (magnification of the original image: ×100). **B** In both cases, the tumor cells were pSTAT3 negative and pSTAT5 positive. In each case, the bottom panel (magnification of the original image: ×400) is a higher-power image of the inset of the upper panel (magnification of the original image: ×100).

Furthermore, one of these two cases stained positive for CD56—indicating that the tumor cells might have derived from NK cells with the *TCRγ* gene in the germline configuration. Despite a relatively high level of Ki67 expression (60%), this individual had much the same tumor cell shape and phenotype as the other cases and was still alive 10 months after diagnosis. Interestingly, a benign NK cell lymphoproliferative disease mimicking GI lymphoma (referred to as NK cell enteropathy) has been reported in humans [23, 24]. Accordingly, the CD56+ cat might have differed from the others and could be classified as having feline LGINKL and not LGITL. However, the etiology of NK cell enteropathy in humans is unknown, and we cannot rule out a common cell origin and/or pathogenic mechanisms in GI-TLPD and NK enteropathy.

CD4/CD8 expression by LGITL cells was only assessed in 11 cases, due to the requirement for frozen biopsies. We observed a variety of phenotypes, including CD4

Table 2 Immunophenotypic findings for 22 cats with LGITL. Quantitative variables are quoted as the median [range].

Immunophenotypic findings	LGITL
CD3 expression (%)	
Lamina propria	91 [20–99], $n = 22$
Epithelium	97 [20–100], $n = 22$
CD20 expression (%)	
Lamina propria	20 [10–40], $n = 22$
Epithelium	5 [1–5], $n = 22$
Ki67 expression (%)	
Lamina propria	30 [7.5–65], $n = 22$
Epithelium	20 [5–45], $n = 22$
pSTAT3 expression (global assessment)	Negative, 22/22
pSTAT5 expression (global assessment)	Positive, 22/22
CD56 expression (global assessment)	Negative, 19/20[a]

[a]CD56 staining was performed on 20 cats.

Table 3 Comparison of clinical and pathological characteristics of human GI-TLPD and feline LGITL [1, 4–7, 26, 27, 29–33].

	Human GI-TLPD	Feline LGITL
Epidemiology	Rare/recently described and validated only in the 2016 WHO Classification	Increasing prevalence over the past decade
Number of cases	57 cases over 25 years	22 cases over 24 months
Age (years), median [range]	51.5 [15–77]	13 [8–16]
Time since onset of clinical signs (days), median [range]	2562 days [122–6.222]	365 days [62–1460]
Male	64%	73%
Clinical signs	Weight loss, diarrhea, vomiting, abdominal pain	Weight loss, vomiting, diarrhea, anorexia
Tumor location	Oral cavity, duodenum, jejunum, ileum, colon, and liver	Duodenum, jejunum, ileum, and liver
Histopathology	Normal or villous blunting	Villous atrophy (86%)
	Monomorphic infiltrate of small lymphocytes in the *lamina propria*	Monomorphic infiltrate of small lymphocytes in the *lamina propria*
IEL	Variable degree of epitheliotropism	Strong epitheliotropism (86 %)
Immunophenotype	CD3+ (100 %)	CD3+ (97%)
	CD4 + /CD8− (56%), CD4−/CD8 + (33%), and CD4−/CD8− (7%) and CD4 + /CD8 + (4%)	CD4+/CD8− (36%), CD4−/CD8+ (27%) and CD4−/CD8− (36%)
	CD56− (100%)	CD56− (95%)
	pSTAT3− (92%)	pSTAT3− (100%)
	pSTAT5 + variable (0 to 44%)	pSTAT5 + (100%)
	Ki67 *lamina propria* < 10%	Ki67 lamina propria 30%
Clonality (small intestine TCR)	Mainly clonal (94%)	Mainly clonal (86%)
Main differential diagnoses	Autoimmune enteropathy, inflammatory bowel disease, refractory celiac disease	Inflammatory bowel disease
Outcome	Indolent course (persistent disease at a median follow up of 5 years)	Indolent course (median survival time of 2 years)

IEL intraepithelial lymphocyte.

single-positive, CD8 single-positive, and CD4− CD8− double-negative profiles—as previously described in human GI-TLPD [1]. Interestingly, CD4−CD8− double-negative tumors are more common in feline disease. However, due to the low number of cases, we failed to detect any correlation between the immunophenotypic profiles and specific presentations, tumor sites, or other pathological features. Further studies are therefore required to establish whether these subtypes of LGITL have different physiopathologic mechanisms.

Several lines of evidence suggest that JAK/STAT signaling is constitutively active in several malignant tumors and has a key role in carcinogenesis. Deregulation of the JAK-STAT pathway is known to regulate lymphocyte development, differentiation, and proliferation, and has recently emerged as a major oncogenic mechanism in several T and NK leukemia and lymphoma subtypes [25]. With regard to GI tract lymphomas, JAK/STAT pathway mutations (in *STAT3*, *STAT5B*, *JAK1*, and *JAK3*) have been reported in 60–70% of cases of MEITL [26–28] and 50% of cases of EATL [29]. Interestingly, the *STAT5B* and *JAK3* mutations reported in MEITL are almost always associated with neutral loss of heterozygosity that preserves the mutated allele—suggesting a key role for the JAK3/STAT5 pathway in MEITL lymphomagenesis [27, 28]. In the context of celiac disease, interleukin-15 (IL-15) promotes IEL survival and accumulation via a cascade involving the phosphorylation of JAK3 and STAT5 [30]. Innate IELs from treatment-refractory patients with celiac disease may acquire gain-of-function mutations in *JAK1* or *STAT3* genes that enhance their response to IL-15 and promote malignant transformation into EATL [31]. It has been recently reported that alterations in the JAK/STAT pathway genes (including *STAT3* mutations, *SOCS1* deletion, and *STAT3-JAK2* fusions) are frequently observed in human GI-TLPD (>80% of cases) [32, 33]. Whereas these alterations supposedly activate the JAK/STAT pathway, the literature data are somewhat discordant. In contrast to the report by Sharma et al. [32], none of the GI-TLPD described by Soderquist et al. [33] (including those with JAK/STAT alterations) showed high levels of pSTAT3 or pSTAT5 expression. These findings need to be clarified in larger studies. Lastly, *JAK3* mutations have been identified in 30% of NK cell enteropathy cases, whereas activation of pSTAT5 was detected in all cases [24]; these findings suggest that mechanisms other than *JAK3*

mutation may activate STAT5 in this subtype. Overall, these data indicate that NK cell enteropathy and GI-TLPD may have some common pathogenic features.

The present study is the first to have screened feline LGITL for the expression of pSTAT3 and pSTAT5. We observed strong expression of pSTAT5 in 100% of the cases, while pSTAT3 expression was low or undetectable. These data may suggest that the STAT5 pathway (rather than the STAT3 pathway) has a key role in indolent feline LGITL.

Interestingly, some reports of cases of human GI-TLPD have mentioned a history of Crohn's disease or autoimmune enteropathy [6, 33]. Accordingly, 10 of the 22 cats (45%) with LGITL showed certain histopathological features of concomitant inflammatory bowel disease, such as prominent or minor cellular polymorphisms with mixed lymphoid and plasmacytic inflammatory infiltrates, neutrophilic cryptitis, and/ or abscesses. Furthermore, the tumor infiltration showed an apical-to-basal gradient in nearly half of the cases, suggesting a process of chronic antigenic stimulation. Taken as a whole, these data prompt us to suggest a new model of lymphomagenesis based on a continuum between inflammatory enteropathy and overt digestive lymphoma. In this model, chronic antigenic stimulation would cause the emergence of low-grade lymphoma within the mucosal compartment (Fig. 4).

An essential goal of comparative genomics in cancer is to define new therapeutic targets that could ultimately be beneficial in both human and veterinary medicine. In this context, JAK/STAT pathway deregulation might be a therapeutic target for feline LGITL and human GI-TLPD. Many JAK-STAT inhibitors are either clinically available (e.g., ruxolitinib) or in preclinical development [25]. Trials in domestic animals are quicker and less restrictive than human studies. Hence, the use of domestic pet species in drug development has expanded dramatically in recent years. This trend is illustrated by the oral pan-tyrosine kinase inhibitor, masitinib, which was developed collaboratively by experts in veterinary and human medicine; the compound's efficacy was first proven in canine mast cell tumors and canine atopic dermatitis [34]. On the same lines, it would be interesting to perform clinical trials of JAK/STAT inhibitors in feline LGITL and then in human GI-TLPD.

Primary intestinal TLPDs are rare in humans and are difficult to diagnose. The validation of a suitable, spontaneous animal model appears to be essential in this context. Feline LGITL is the most frequent gastrointestinal tract cancer in this species; it shares clinical, histological, immunohistochemistry, and clonality features with human GI-TLPDs. The robust data generated here (owing notably to innovative histopathologic and immunohistochemical experiments and the dual pathology assessment by both veterinary and clinical specialists) enabled us to validate a feline model of GI-TLPD. Further studies of the molecular pathogenesis of LGITL (with the objective of providing new insights into human GI-TLPD) are now warranted. To

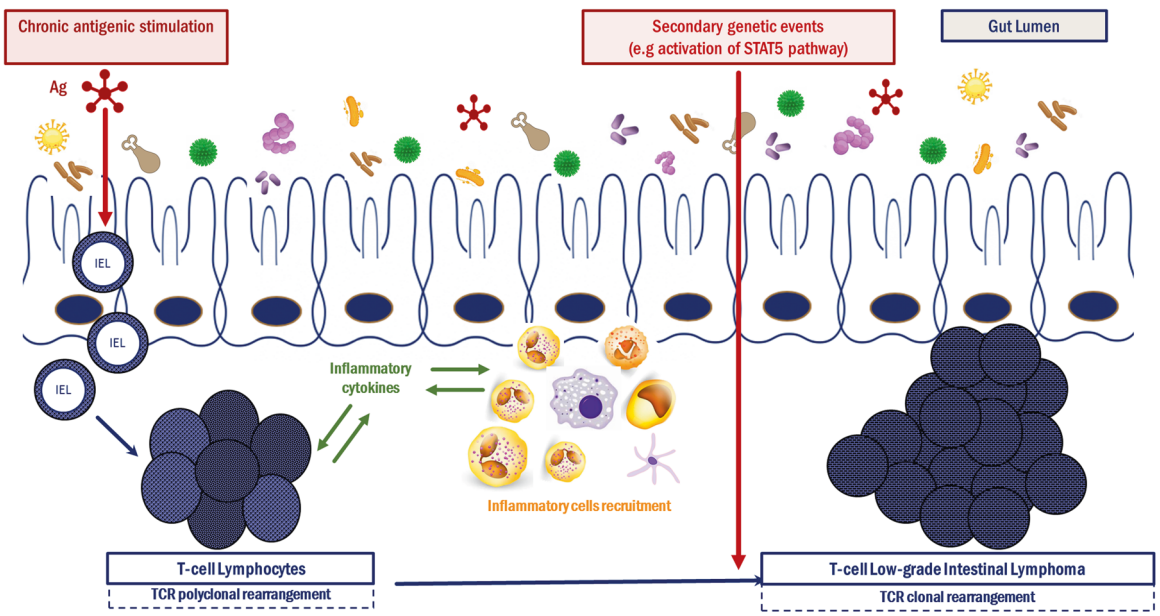

Fig. 4 A new model of lymphomagenesis in indolent GI-TLPD. As suggested by the observed apical-to-basal gradient, the initiating event would be chronic stimulation by a food antigen or an antigen from a viral or bacterial pathogen. This would trigger the expansion of antigen-specific CD4 and/or CD8 T cell lymphocytes, cytokine production, the recruitment of immune and inflammatory cells (monocytes, neutrophils, plasmocytes, macrophages, etc.), and thus T cell proliferation. Lastly, additional genetic and/or epigenetic events (e.g., JAK/STAT pathway deregulation, leading to STAT5 activation) would result in the emergence of T cell clones and T cell transformation and expansion.

date, a "watch and wait strategy" or low-dose methotrexate chemotherapy (in the event of severe symptoms) is recommended in human patients because a better treatment regimen has not emerged from earlier studies. Identifying new "driver" events in feline LGITL might be very helpful for developing targeted therapies and improve the quality of life in human patients with GI-TLPD. Lastly, characterizing the genetic landscape of LGITL might provide relevant data for human GI-TLPDs and—in line with the "one health" concept [35]—might pave the way for the more widespread use of spontaneous pet models in oncology.

Data availability

The authors confirm that all data generated or analyzed during this study are included in this published article and its Supplementary information files.Author contributions VF, LC, JB, and OH initiated, designed, and supervised the study. VF included patients, collected clinical and laboratory data, and assisted surgeons with intestinal biopsies. JB, HH, and NC conducted histopathological analyses. MET performed clonality analysis. JB, TJM, MET, EM, HH, and NC interpreted histology, immunohistochemistry, and clonality data. VF, MVP, HH, LC, JB, and NC analyzed the data and drafted the manuscript. VF, LC, HH, MVP, GM, TJM, NC-B, MET, EM, NC, OH, and JB reviewed the manuscript. All the authors read and approved the final version.

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Compliance with ethical standards

Ethics approval This study was approved by the investigational review board at Alfort National Veterinary School (COMERC, Maisons-Alfort, France; reference: 2017-05-09).

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