INVESTIGATIONS OF A NOVEL LYMPHOPROLIFERATIVE DISEASE IN BRITISH SHORTHAIR KITTENS

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ABSTRACT

In 2009, three sibling British shorthair (BSH) kittens presented with lymphoproliferative disease (LPD) causing massive enlargement of multiple lymph nodes, a presentation that suggested an inherited predisposition to the disease. While aspects of the disease presentation suggested a diagnosis of lymphoma, other features were inconsistent with lymphoid neoplasia. In particular, the consistently young age of affected kittens, the pattern of disease affecting multiple littermates, and the presence of such marked generalised lymphadenopathy, were all atypical for feline lymphoma. This unusual constellation of clinical and pathologic features in affected BSH kittens had not been previously reported in cats but had several similarities to the human disease autoimmune lymphoproliferative syndrome (ALPS), a rare inherited disorder causing persistent LPD, increased numbers of CD3+/CD4-/CD8- double negative T-cells (DNT cells) and variable manifestations of autoimmunity. The majority of human ALPS patients have inherited *Fas* gene mutations causing defective T-cell apoptosis, although in some patients the cause of disease is still unknown.

The thesis further describes and investigates this novel LPD in BSH kittens. The results of breeding trials, pedigree information and reviews of historical records support an inherited basis for the disease, most likely with either a simple autosomal recessive or modified autosomal dominant mode of inheritance. The typical clinical presentation is the development of a massive multicentric lymphadenopathy, splenomegaly and probable haemolytic anaemia in previously healthy kittens between 5 to 7 weeks of age. Microscopic pathology and immunophenotypic studies are suggestive of multicentric T-cell lymphoma affecting the lymph nodes, spleen, and sometimes other organs, but clonality assays confirm a non-clonal and likely non-neoplastic T-cell LPD. Where tested, the proliferating T-cells show a DNT cell immunophenotype and reduced apoptosis on *in situ* methods. Qualitative *Fas* gene abnormalities were not identified in affected kittens using reverse-transcriptase polymerase chain reaction techniques.

The studies described in the thesis therefore confirm a novel and likely non-neoplastic T-cell LPD in BSH kittens with a probable inherited basis. Results support defective T-cell

apoptosis as a possible factor in disease development, although causative genetic abnormalities have not yet been identified. The disease in kittens has several similarities to ALPS in people, although the apparent absence of *Fas* gene abnormalities in affected kittens may limit the use of the feline disease as a disease model for ALPS.

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ABBREVIATIONS

AAFCO	American Association of Feed Control Officials
AI	apoptotic index, a measure of the level of apoptosis present within the relevant tissue field(s) using the number of apoptotic cells as the basis for calculation
ALL	acute lymphoblastic leukaemia
ALPS	autoimmune lymphoproliferative syndrome, a human disease of defective lymphocyte apoptosis resulting in lymphoproliferation including lymphadenopathy and splenomegaly and variable manifestations of autoimmunity
ALPS-CASP10	People fulfilling the diagnostic criteria for autoimmune lymphoproliferative syndrome (ALPS) with either germline or somatic mutations in the caspase 10 (*CASP10)* gene
ALPS-FAS	People fulfilling the diagnostic criteria for autoimmune lymphoproliferative syndrome (ALPS) with either heterozygous or homozygous germline mutations in the *Fas* gene
ALPS-FASLG	People fulfilling the diagnostic criteria for autoimmune lymphoproliferative syndrome (ALPS) with germline mutations in the *Fas ligand* gene
ALPS related disease	Diseases in people with similar clinical signs and pathology to autoimmune lymphoproliferative syndrome (ALPS) but not meeting the diagnostic criteria for ALPS. ALPS-related disorders include caspase-8 deficiency state, RAS-associated autoimmune leukoproliferative disease, Dianzani autoimmune lymphoproliferative syndrome and X-linked lymphoproliferative syndrome.
ALPS-sFAS	People fulfilling the diagnostic criteria for autoimmune lymphoproliferative syndrome (ALPS) with somatic mutations in the *Fas* gene
ALPS-U	People fulfilling the diagnostic criteria for autoimmune lymphoproliferative syndrome (ALPS) without a determined genetic defect
ANOVA	analysis of variance
AR	apoptotic rate, a measure of the level of apoptosis present within the relevant tissue field(s) using apoptotic area as the basis for calculation
AT	ataxia-telangiectasia
ATM	the ataxia telangiectasia mutated gene
BSH	British shorthair breed of cat
CASP10	the caspase 10 gene
CBC	complete blood count

CD	cluster of differentiation
CD3	cluster of differentiation antigen 3, a protein complex associated with the T-cell receptor (TCR) complex on mature T-cells and composed of 4 distinct chains (γ, δ and two ε chains)
CD4	cluster of differentiation antigen 4, a glycoprotein predominantly expressed by and on the surface of helper T-cells
CD8	cluster of differentiation antigen 4, a transmembrane glycoprotein predominantly expressed by and on the surface of cytotoxic T-cells usually composed of α and β chains
CMA	chromosomal microarray analysis
Coombs test	blood test used to detect the presence of antibodies to erythrocytes and providing evidence of immune-mediated erythrocyte destruction and immune-mediated haemolytic anaemia
ConA	*Concanavalia ensiformis* type IV-S
CRT	capillary refill time
CTLA-4	cytotoxic T-lymphocyte antigen 4
CVID	combined variable immunodeficiency
Cy5	cyanine 5, a fluorescent dye used in flow cytometry
DAB	3,3-diaminobenzidine, a chromagen used in immunohistochemistry
DMEM	Dulbecco's Modified Eagle Medium
DN	double negative, lymphocyte development stages expressing neither CD4 nor CD8 cell surface proteins, and including the thymic DN1, DN2, DN3 and DN4 stages of T-cell development
DNA	deoxyribonucleic acid
DNT cell	double negative T-cell) a CD3+ T-cell expressing neither CD4 nor CD8 cell surface markers
DP	double positive, lymphocyte development stages expressing both CD4 and CD8 cell surface proteins
DSH	domestic shorthair breed of cat
dUTP	deoxyuridine triphosphate
EBV	Epstein-Barr virus (alternatively termed human herpesvirus-4 (HHV-4))
EDTA	ethylenediaminetetraacetic acid
ELISA	enzyme-linked immunosorbent assay
F	inbreeding co-efficient, calculated as a percentage of the chances that two alleles in offspring will be identical by descent
FACS	fluorescence activated cell sorting
FADD	Fas-associated death domain protein
Fas	The Fas protein

Fas	the Fas gene (also termed CD95, Apo1, Apt and TNFRSF6 (tumour necrosis factor receptor superfamily member 6)
FasL	the Fas ligand protein
FasL	the Fas ligand gene
FeLV	feline leukaemia virus
FFPE	formalin-fixed paraffin-embedded
FITC	fluorescein isothiocyanate
FIV	feline immunodeficiency virus
FOCMA	feline oncornavirus cell membrane antigen
FSC	forward scatter
Gld	the generalised lymphoproliferative disease gene, one of the genes mutated in the mice with lymphoproliferative disease resembling autoimmune lymphoproliferative syndrome (ALPS) in people
H&E	haematoxylin and eosin
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HHV-4	human herpesvirus-4, alternatively termed Epstein Barr virus (EBV)
HHV-8	human herpesvirus-8, alternatively termed Kaposi 's sarcoma-associated virus
HIV	human immunodeficiency virus, the causative agent of acquired immunodeficiency syndrome (AIDS) in people
HNPCC	hereditary non-polyposis colorectal cancer
hpf	high power field
HRP	horseradish peroxidase
HSC	haematopoietic stem cell
HTLV-1	human T-cell leukaemia virus-1 type 1
IGH	immunoglobulin heavy chain locus, a gene present in B-cells that undergoes rearrangement early in B-cell development and used to assess B-cell clonality in lymphoid proliferations
IL	interleukin, one of a group of cytokines involved in a wide range of signalling processes
IFA	immunofluorescence antibody
LGL	large granular lymphocyte, a subset of lymphocytes characterised by intracytoplasmic azurophilic granules, and which in humans may be of natural killer (NK)-cell (CD3–) or mature T-cell (CD3+) lineage
LPD	lymphoproliferative disease (any disease, either neoplastic and non-neoplastic, in which abnormally excessive numbers of lymphocytes are produced)
Lpr	the lymphoproliferation gene, one of the genes that mutated in the mice with lymphoproliferative disease resembling autoimmune lymphoproliferative syndrome (ALPS) in people

lymphoma	a clonal proliferation of lymphocytes (lymphoid neoplasia)
lymphoblast	large lymphocyte
MAA	morphometric area analysis, used to calculate the apoptotic rate (AR) within tissue fields, determined by calculating the area of the relevant field(s) showing evidence of apoptosis (positive staining)
MALT	mucosa-associated lymphoid tissue, aggregations of lymphoid tissue at various mucosal sites throughout the body, particularly the gastrointestinal tract
MCC	morphometric cell count, used to calculate the apoptotic index (AI) within tissue fields, determined by counting the number of cells within the relevant field(s) showing evidence of apoptosis (morphology and positive staining)
MHC	major histocompatibility complex
MLH1	MutL homolog 1 protein, encoded by the *MLH1* gene
MLH1	the MLH1 gene, one of the mismatch repair (MMR) genes, defects in which are associated with hereditary non-polyposis colorectal cancer (HNPCC) in people
MMR	mismatch repair, a highly conserved system within prokaryotes and eukaryotes that recognises and repairs errors in base insertion and deletion during DNA replication and recombination and repairs DNA damage
MMR	the mismatch repair genes, particularly including *MLH1*, *MSH2* and *MSH6*
mRNA	messenger ribonucleic acid
MSH2	MutS protein homolog 2 protein, encoded by the *MSH2* gene
MSH2	the MSH2 gene, one of the mismatch repair (MMR) genes, defects in which are associated with hereditary non-polyposis colorectal cancer (HNPCC) in people
MSH6	mutS homolog 6 protein, encoded for by the *MSH6* gene
MSH6	the MSH6 gene, one of the mismatch repair (MMR) genes, defects in which are associated with hereditary non-polyposis colorectal cancer (HNPCC) in people
NBS	Nijmegen breakage syndrome
NK cells	natural killer cells, a type of cytotoxic lymphocyte distinct from B-cells and T-cells, which in people usually express surface markers CD16 and CD56
NK T-cells	natural killer T-cells, a heterogeneous subset of T-cells with properties of both T-cells and natural killer (NK) cells
NMD	nonsense-mediated decay (of RNA)
NRAS	the neuroblastoma RAS gene
nRBCs	nucleated (immature) erythrocytes, typically metarubricytes
NZVP	New Zealand Veterinary Pathology

PALS	periarteriolar lymphoid sheath, a cuff of lymphocytes (mainly T-cells) surrounding small splenic arterioles and part of the splenic white pulp
PBMCs	peripheral blood mononuclear cells (includes lymphocytes and monocytes)
PBS	phosphate buffered saline
PCR	polymerase chain reaction
PCV	packed cell volume
PE	phycoerythrin
PEL	primary effusion lymphoma, an uncommon B-cell lymphoma typically presenting as an effusion without a tumour mass or nodal involvement, and most commonly associated with human herpes virus 8 (HHV-8) and human immunodeficiency virus (HIV) infection in people
PTGC	progressive transformation of germinal centres
Q1	British shorthair-Manx cross queen, dam of LPD-affected Litters 1 and 2
Q2	British shorthair queen, dam of LPD-affected Litter 0 and granddaughter of T1
qPCR	real-time polymerase chain reaction
REAL	Revised European/American Lymphoma classification
RIPA	radio-immunoprecipitation assay
RNA	ribonucleic acid
RT	room temperature
RT-PCR	reverse transcriptase polymerase chain reaction
SCID	severe combined immunodeficiency
SLE	systemic lupus erythematosus
SSC	side scatter
T1	British shorthair tom; sire of LPD-affected Litters 1 and 2, and both grand-sire and great-grand sire of LPD-affected Litter 0
T2	Oriental tom; sire of unaffected Litter 3
T3	Unknown tom; sire of Litter 4
T4	Unknown tom; sire of Litter 5
T5	British shorthair tom; sire of LPD-affected Litter 0 and son of T1
T6	British shorthair tom; common ancestor off all LPD-affected litters
TBS	tris-buffered saline
TCR	T-cell receptor
TCRαβ	a T-cell receptor composed of α and β protein chains
TCRγδ	a T-cell receptor composed of γ and δ protein chains

TCRG	the T-cell receptor γ locus, a gene present in T-cells that undergoes rearrangement early in T-cell development, and used to assess T-cell clonality in lymphoid cell proliferations
TdT	terminal deoxynucleotidyl transferase
TNF	tumour necrosis factor
TUNEL	terminal deoxynucleotidyl transferase dUTP nick end labelling, a method for used to detect the DNA fragmentation typically present when cells undergo apoptosis by labeling the terminal ends of nucleic acids
WAS	Wiskott-Aldrich Syndrome
WHO	World Health Organization
XLP	X-linked lymphoproliferative disease
χ^2	chi-square