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CASE INFORMATION:

Lilly, a 4 ½-year-old female spayed Old English Mastiff, presented to the Purdue University Veterinary Teaching Hospital emergency service on referral from her primary veterinarian (rDVM). Thrombocytopenia, elevated liver enzymes (ALT: 1294, RI: 3-69; ALP: 1214, RI: 20-157; GGT: 26, RI: 5-16), total bilirubin (T. bili: 5.20, RI: 0.10-0.80), and increased respiratory rate were the primary abnormalities identified by the rDVM. Upon initial examination, her temperature was elevated (103.4°F), and the popliteal, prescapular, and facial lymph nodes were enlarged. A CT scan revealed hepatosplenomegaly, mild sternal lymphadenopathy, and a small amount of peritoneal effusion.

LABORATORY DATA:

HEMOGRAM RESULTS

TEST	RESULT 4/25/19	RESULT 4/26/19	REFERENCE INTERVAL	UNITS
HCT	37.1	**27.1**	37.0-55.0%	%
RBC	**5.27**	**2.84**	5.5-8.5	M/µL
Hb	12.3	**9.0**	12.0-18.0	g/dL
MCV	70.4	70.5	60.0-75.0	fl
MCHC	33.3	33.2	32.0-36.0	g/dL
RETIC	34.4	36.3	<100	K/µL
Total protein	**5.5**	**4.6**	6.8-8.0	g/dL
WBC	**4.6**	**2.7**	6.0-17.0	K/µL
Segmented Neutrophils	**2.7**	**1.4**	3.0-12.0	K/µL
Band Neutrophils	0.09	0.22	0.00-0.30	K/µL
Lymphocytes	**0.3**	**0.2**	1.0-5.0	K/µL
Monocytes	0.32	**0.08**	0.15-1.35	K/µL
Large granular lymphocytes**	**1.24**	**0.76**	0.00-0.00	K/µL
Platelets	**21**	**17**	200-500	K/µL

**Figure 1

SERUM CHEMISTRY RESULTS

TEST	RESULT 4/26/19	REFERENCE INTERVAL	UNITS
Glucose	**61**	67-132	Mg/dL
Blood Urea Nitrogen	29	7-32	Mg/dL
Creatinine	1.00	0.50-1.50	Mg/dL
Phosphorus	4.9	2.2-7.9	Mg/dL
Calcium	**7.9**	9.7-12.3	Mg/dL

Sodium	**135**	138-148	Mmol/L
Potassium	4.1	3.5-5.0	Mmol/L
Chloride	107	105-117	Mmol/L
Carbon Dioxide	15	13-24	Mmol/L
Anion Gap	17.1	9.0-18.0	Mmol/L
Total Protein	**4.3**	4.8-6.9	g/dL
Albumin	2.4	1.7-3.8	g/dL
Globulin	1.9	1.7-3.8	g/dL
A/G ratio	1.3	0.8-1.9	--
ALT	**1294**	3-69	IU/L
Alkaline Phosphatase	**1214**	20-157	IU/L
GGT	**26**	5-16	IU/L
Total bilirubin	**5.20**	0.10-0.80	Mg/dL
Cholesterol	**105**	125-301	Mg/dL
Amylase	439	378-1033	IU/L
Lipase	178	104-1753	IU/L
Fibrinogen	144	69-289	Mg/dl

ADDITIONAL TESTING:

TEST	RESULT 4/26/19	REFERENCE INTERVAL	UNITS
Ammonia	<8.7	1.0-46.0	µmol/L
4Dx***	Negative	Negative	Negative

*** *A. Phagocytophilum/ A. platys, B. burgdorferi, D. immitis, E. canis/E. ewingii*

CYTOLOGIC IMAGES:

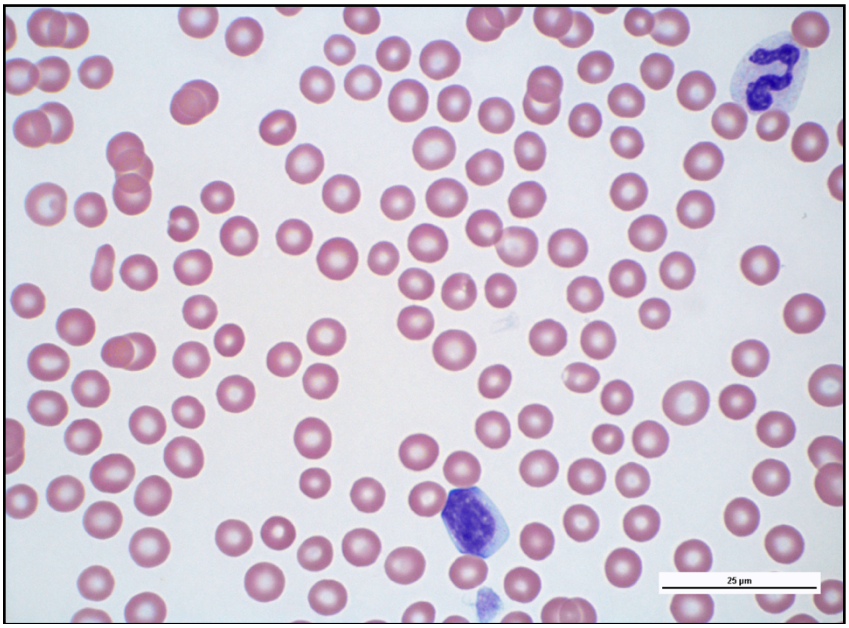

FIGURE 1. Blood Smear, 100x. Modified Wright's Stain. Large granular lymphocyte: this photomicrograph represents a mononuclear cell with a moderate amount of pale blue cytoplasm containing small, dusty pink granules and a moderately clumped chromatin pattern.

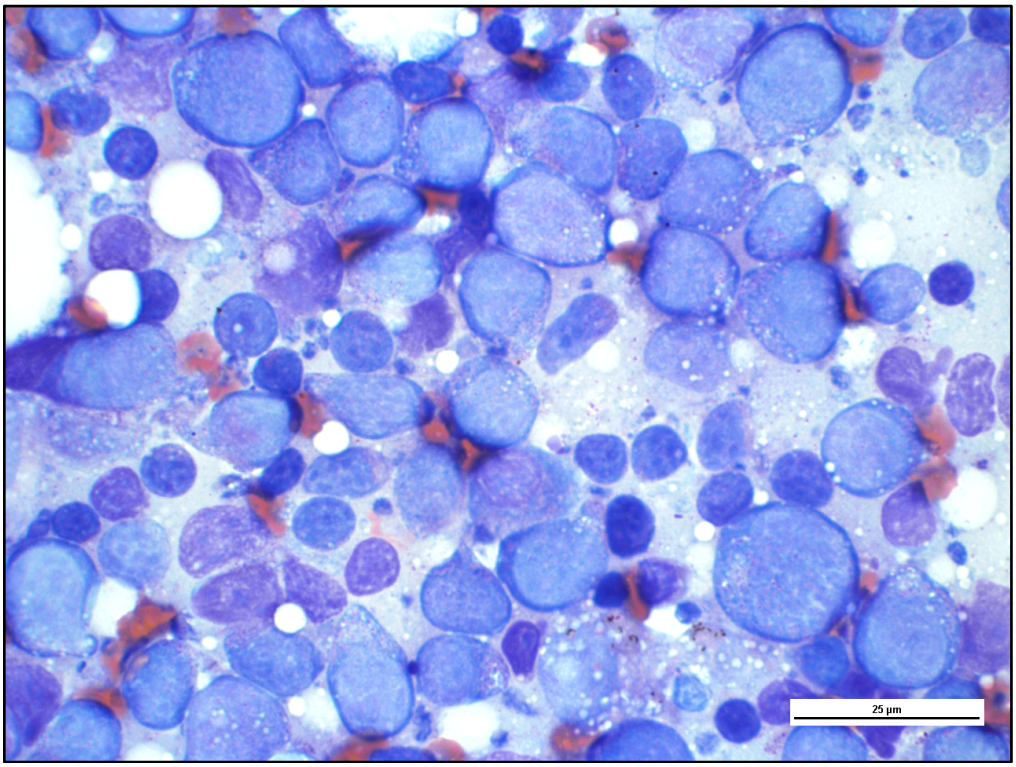

FIGURE 2. Lymph node aspirate, 100X. Modified Wright's Stain. The lymphoid population consisted of high numbers of intermediate to large lymphocytes. The lymphoid cells had small to moderate amounts of basophilic cytoplasm that often contained a dusting of pink granules. Their nuclei had a moderately clumped to finely stippled chromatin pattern and occasionally contained one to two medium-sized prominent nucleoli.

ADDITIONAL FINDINGS: A bone marrow aspirate and core biopsy were evaluated. These preparations were highly cellular and of excellent diagnostic quality. Both the aspirate and core displayed moderate myeloid and erythroid hypoplasia along with ineffective thrombopoiesis. On the bone marrow core, there was evidence of bone remodeling and mild osteosclerosis. In addition, a prominent population (~15% of all nucleated cells) were large granular lymphocytes (LGLs), morphologically similar to the LGLs from the peripheral blood and lymph nodes (Figure 3). A histiocytic population was also present; these often displayed erythroid phagocytosis, and less frequently, myeloid phagocytosis (Figure 3 and Figure 4).

Immunohistochemistry was evaluated on the bone marrow core, the round cells were strongly positive for CD18 and CD3, and there was scattered immunoreactivity for CD11d (Figure 5). Further evaluation via immunocytochemistry revealed only scattered lymphocytes positive for either CD4 or CD8α.

PCR FOR ANTIGEN RECEPTOR REARRANGEMENT (PARR):
Bone marrow was submitted for PARR analysis and displayed a clonally rearranged of the T cell receptor gamma gene.

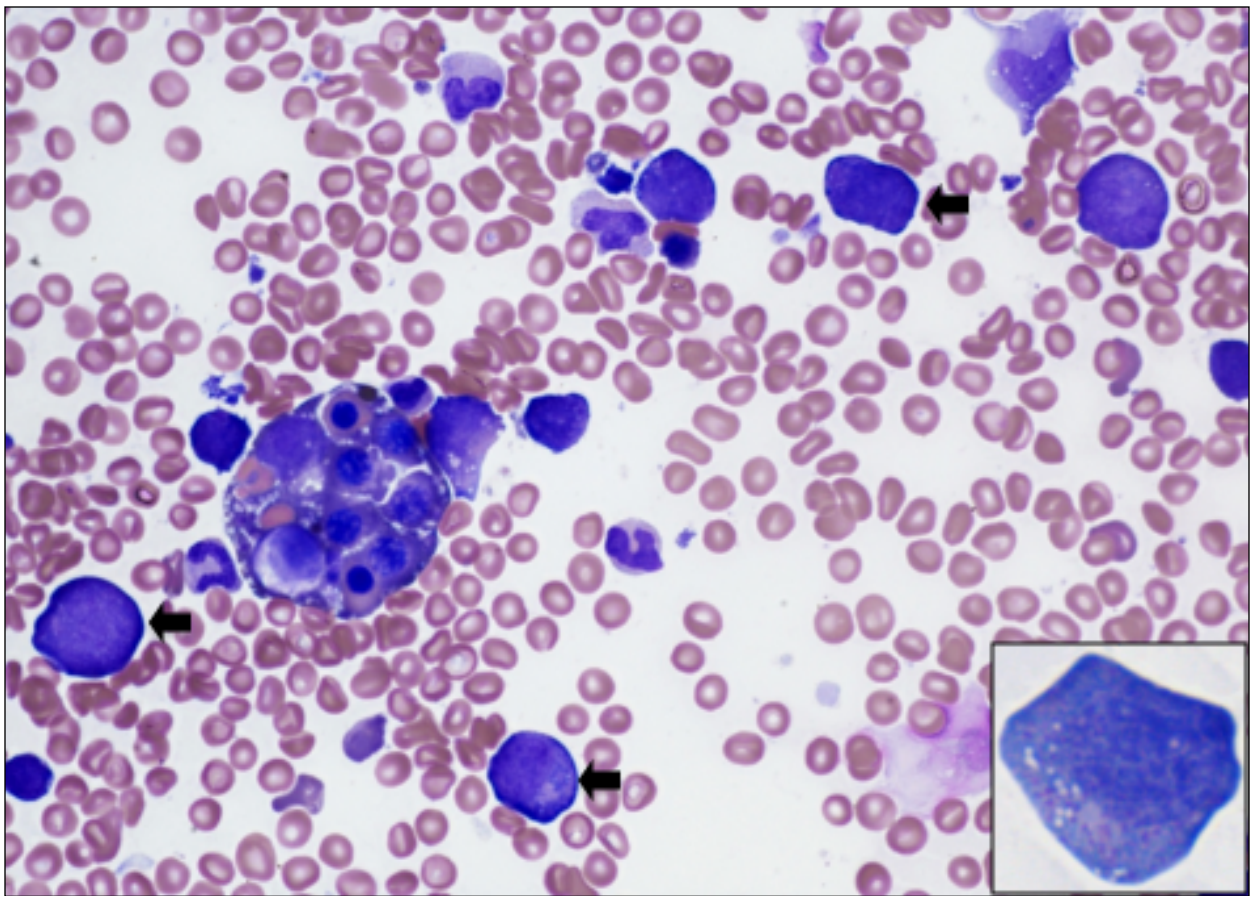

Figure 3. Bone marrow aspirate, 100x. Modified Wright's stain. Several large granular lymphocytes (arrows) and a macrophage displaying phagocytosis of erythroid precursor cells, mature erythrocytes, and a single leukocyte are noted. **Inset:** An enlarged image of the large granular lymphocyte to highlight the granularity of the cell.

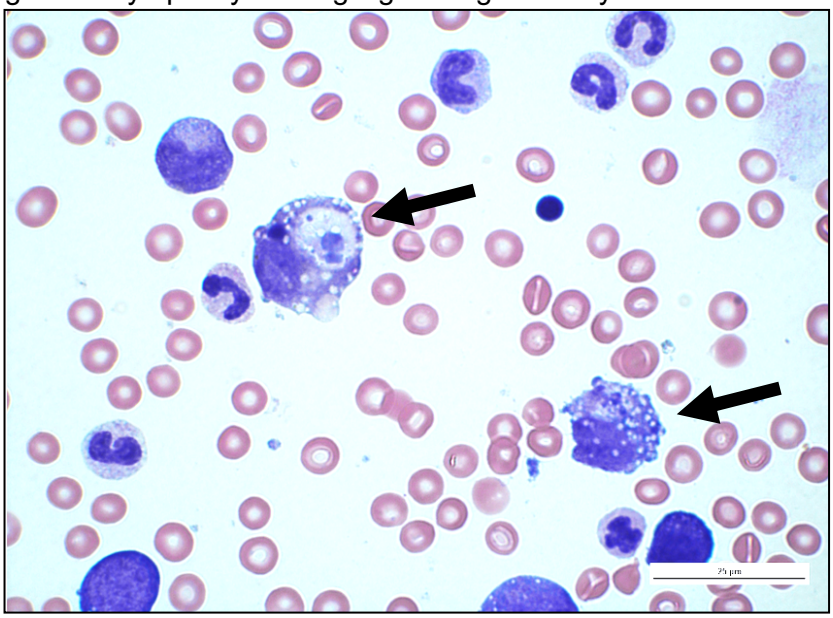

Figure 4. Bone marrow aspirate, 100x. Modified Wright's stain. Low numbers of histiocytes displaying phagocytosis are seen (arrows).

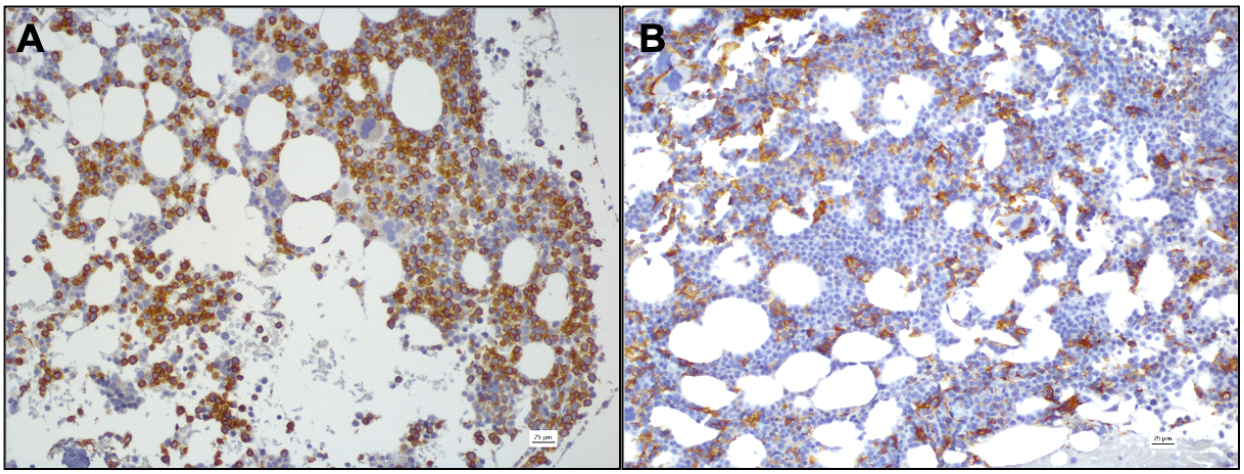

Figure 5. Bone marrow core, 20X. A. IHC for CD3 showing marked immunoreactivity by a population of lymphocytes widely spread throughout the bone marrow space. **B.** IHC for CD11d showing scattered positive cells.

QUESTIONS:

1. What is the cause of the prominent phagocytosis of both myeloid and erythroid precursor cells in this case?

2. Large granular lymphocytes can belong to what distinct cell types, and how might this information direct additional testing to characterize the neoplastic LGLs in this case?