Spontaneous Pathology of the Lymphoid and Hematopoietic System of Crl:CD-1 (ICR) mice

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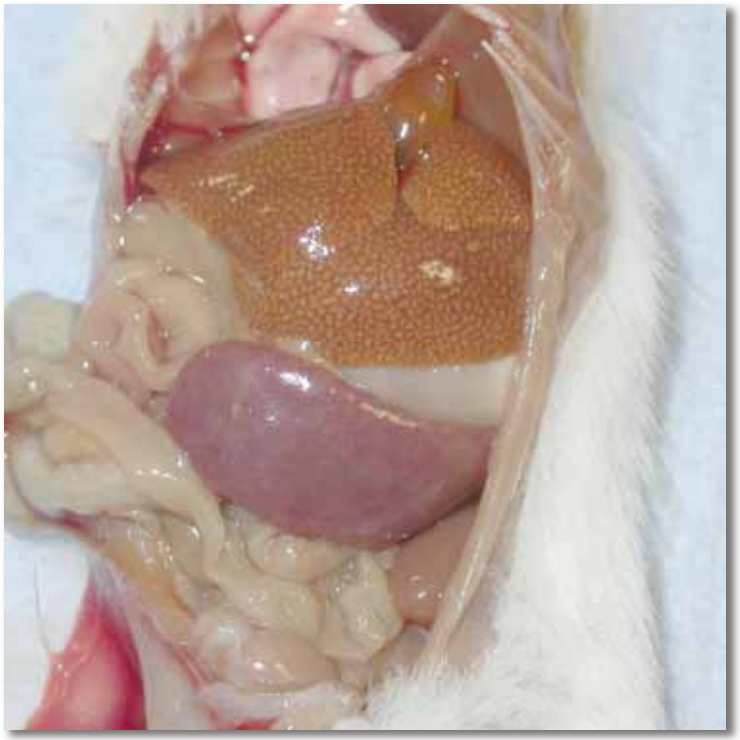
Introduction

- Neoplastic and Non-neoplastic lesions recorded in Crl:CD-1 (ICR) mice at Charles River , PCS-Edinburgh

- Thymus
- Spleen
- Mesenteric Lymph Node



Methods

- Fixed in neutral buffered formalin
- Lesions visible by H&E stains

- Historical Control Data (6 years) male and female Crl:CD-1 mice :
  - 104 week studies – 1658 mice
  - 80 Week studies – 608 mice
  - 13 week studies – 294 mice

THYMUS

Thymus Findings

- Findings reported in eight 104 week studies:

Hyperplasia	(183/1658)
Arteritis/periarteritis	(61/1658)
Lymphoid depletion	(13/1658)
Lymphocytolysis	(13/1658)
Plasmacytosis	(9/1658)

- Tubular cystic hyperplasia, Pigmented macrophages (each 7/1658)
- Increased macrophages, Thymoma, Focal necrosis (each 3/1658)
- Amyloid (2/1658)
- Ectopic thyroid/parathyroid, Haemangiosarcoma, Thrombus (each 1/1658)

Thymus Findings

- Findings reported in five 80 week studies:

  - Lymphoid depletion (155/608)
  - Hyperplasia (89/608)
  - Arteritis/periarteritis (13/608)

  - Tubular cystic hyperplasia, Cyst, Thymoma (each 4/608)

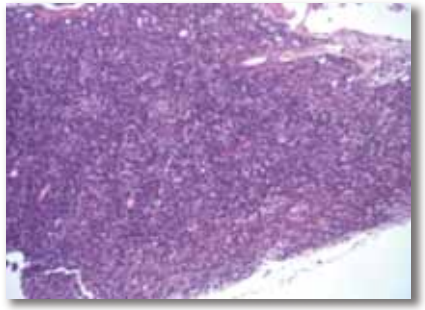
Thymus Findings

- Findings reported in fourteen 13 week studies:

Lymphoid depletion	(7/294)
Hyperplasia	(2/294)
Plasmacytosis	(1/294)
Pigmented macrophages	(1/294)
Tubular cystic hyperplasia/Cyst	(1/294)

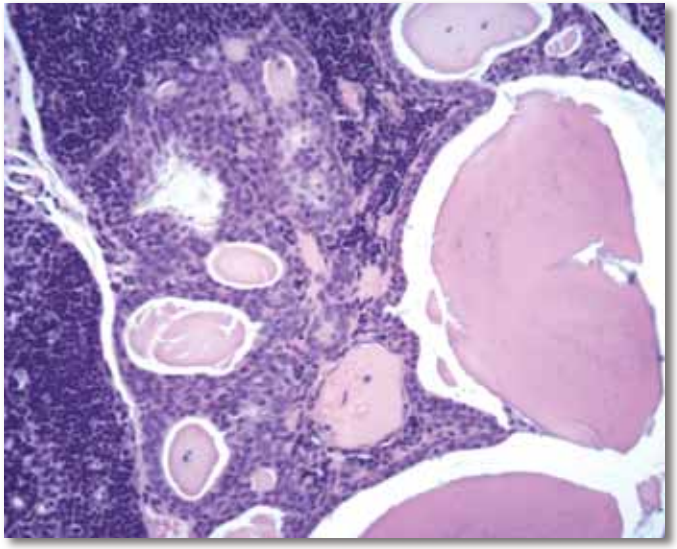
Thymus Findings

- Lymphoid depletion (decreased cellularity, atrophy, involution)
  - Medullary and cortical elements affected
  - Variable in grade and incidence
  - Adipocyte infiltration of connective tissue capsule and septae
  - Increased incidence with age, especially males
  - Exacerbated by stressors; commonly recorded in “unscheduled sacrifice” animals
  - Medullary epithelial structures form cords/ribbons; may progress to focal/diffuse tubular cystic hyperplasia
  - Hyperplastic medullary elements prominent with lymphoid atrophy



Thymus Findings

- Epithelial Cysts (Tubular Cystic Hyperplasia)
  - Tubules filled with eosinophilic colloid
  - Cysts lined by cuboidal or columnar cells, may be ciliated
  - More prominent in medulla

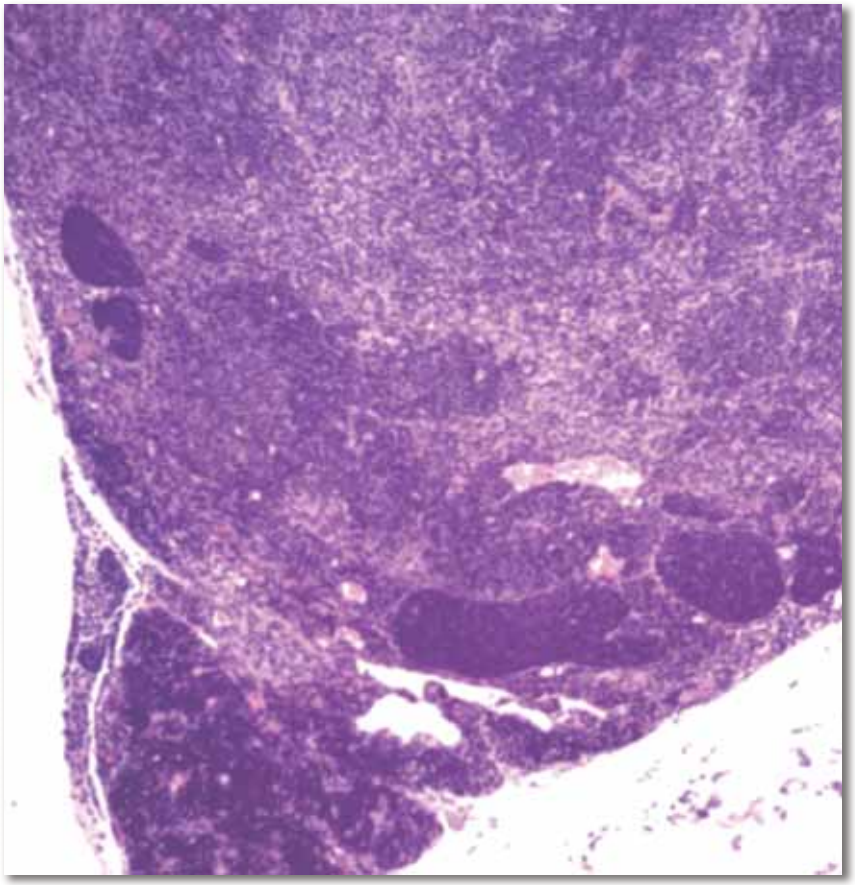


Thymus Findings

- Hyperplasia (mixed)
  - Enlarged thymus macroscopically
  - Proliferation of both lymphoid and/or epithelial elements
  - Ageing change, starts c 60 weeks age
  - Tends to be more common in females, and mainly lymphoid elements
  - Normal architectural arrangements remain, but may have patchy atrophy in cortex
  - Benign - Does Not Progress To Lymphoma
  - Differential Diagnosis: lymphoma - which obliterates normal architecture

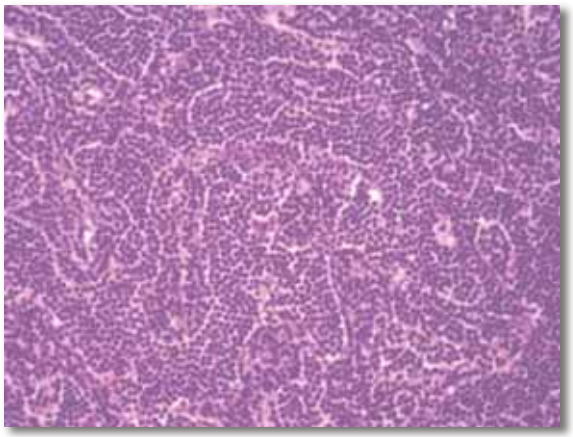
Thymus Findings

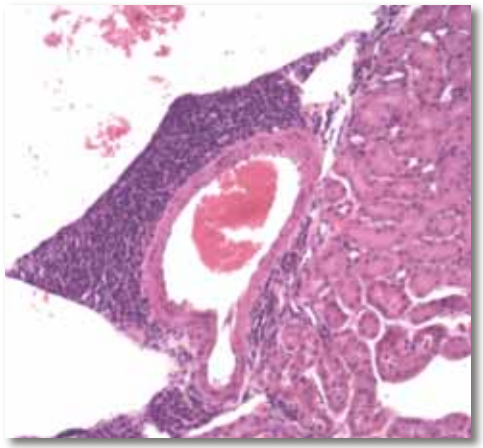
- Hyperplasia
  - Mainly affects medulla and seems to be hormonally mediated
  - B-cell aggregates +/- germinal centres as a focal or diffuse lesion
  - atypical T-cell hyperplasia
  - Benign - Does Not Progress To Lymphoma



Thymus Findings

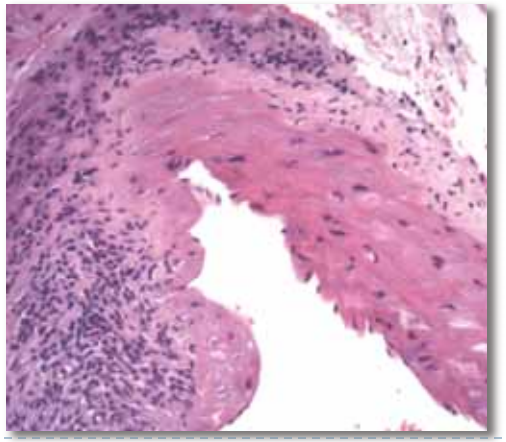
- Hyperplasia - sequelae
  - "Lymphoid overspill" occurs as sheets of non-neoplastic lymphocytes
  - Affects liver, mediastinum, periovarian tissues, periuterine tissue, renal pelvis, lacrimal and salivary gland





Thymus Findings

- Arteritis/periarteritis
  - Ageing change, rare in mice less than 6 months old
  - Predominantly in females
  - 104 week study incidence: 3.6% thymus, 0.5% spleen, 0.5% mesenteric LN
  - Common in animals with concurrent high grade nephropathy
  - Walls of small and large arteries
  - Fibrinoid necrosis of smaller arterioles
  - Can lead to thrombosis of vessel
  - Also affects spleen and lymph nodes



SPLEEN

Spleen Findings

- Findings reported in eight 104 week studies:
  - Increased extramedullary hematopoiesis (278/1658)
  - Hyperplasia (82/1658)
  - Red pulp hyperplasia (77/1658)
  - Lymphoid depletion (18/1658)
  - Arteritis/periarteritis (8/1658)
  - Decreased cellularity of red pulp (5/1658)
  - Hemangiosarcoma (4/1658)
  - Lymphocytolysis, Hemangioma (each 3/1658)
  - Plasmacytosis, Capsular fibrosis, Angiectasis (each 2/1658)
  - Focal necrosis (1/1658)

Spleen Findings

- Findings reported in five 80 week studies:

Increased extramedullary hematopoiesis	(196/608)
Hyperplasia	(52/608)
Lymphoid depletion	(6/608)
Hemangiosarcoma	(6/608)
Arteritis/periarteritis	(1/608)

Spleen Findings

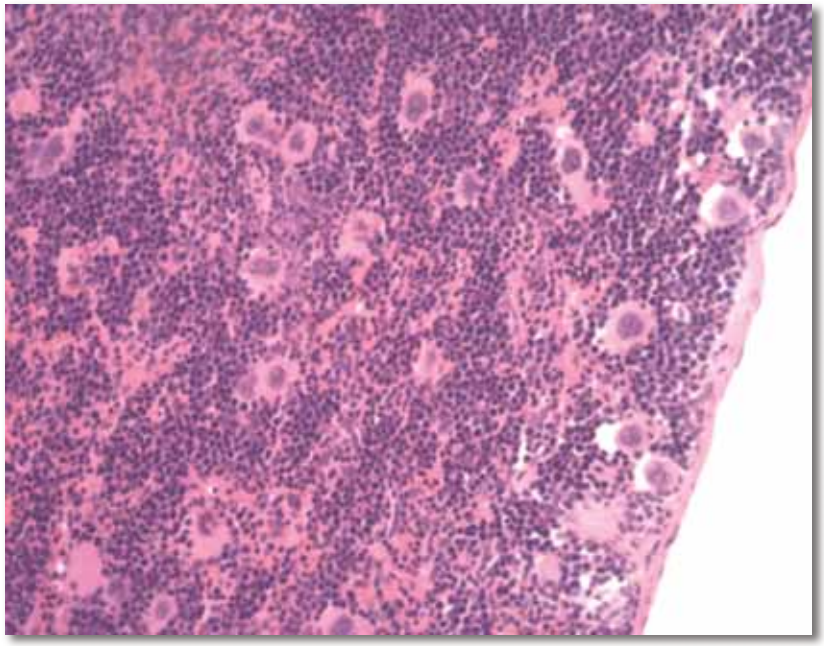
- Findings reported in fourteen 13 week studies:
  - Increased extramedullary hematopoiesis (39/294)
  - Megakaryocytosis (5/294)
  - Increased hemosiderin (2/294)
  - Hyperplasia (1/294)

Spleen Findings

- Increased Extramedullary Hematopoiesis
  - Most common finding recorded in CD-1 mice of any age
  - Gender related: grades normally higher in females than males
  - “Called” when grade in male mice ≥ female controls
  - Necropsy: spleens enlarged, red, firm
  - Often accompanied by pigmented macrophages (haemosiderosis) and megakaryocytosis in LN
  - May be exacerbated by some types of anemia, inflammation, blood sampling
  - Commonly recorded in “unscheduled sacrifice” animals
  - Need to differentiate from early myeloid leukemia

Spleen Findings

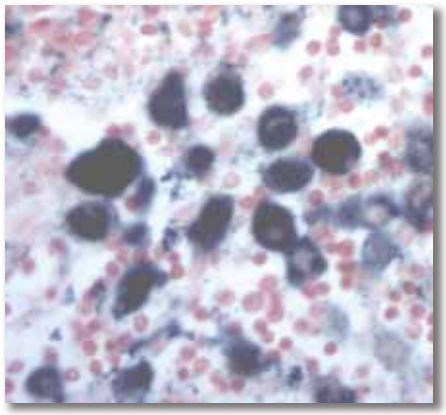
- Normal amount of EMH with increased numbers of megakaryocytes in female control CD-1 mouse



Spleen Findings

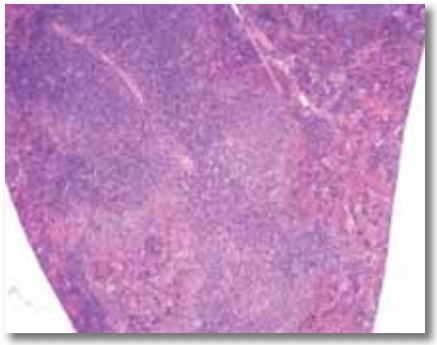
- Amyloid accumulation
  - Ageing change, variable in grade and incidence
  - Used to be commonly reported, but since 1990s rarely reported in European Crl: CD-1

- Pigmented macrophages
  - Increased Haemosiderin deposition (PPB)
  - Higher grades in females is normal
  - Incidence increases with age
  - “Called” when male animal spleens look similar to “normal” female levels



Spleen Findings

- Lymphoid Hyperplasia
  - Increase in size or components of T-cell, B-cell zones or PALS
  - Bulging of splenic capsule; pale foci at necropsy
  - Focal, multifocal or diffuse
  - Enlarged secondary follicle caused by systemic immune stimulation and common in gavage or intra-peritoneal dosed animals
  - Differential Diagnosis: T-cell zones become enlarged with early lymphoma/leukemia



LYMPH NODES

Mesenteric Lymph Node Findings

- Findings reported in eight 104 week studies:
  - Sinus dilation (91/1658)
  - Increased extramedullary hematopoiesis (67/1658)
  - Angiomatous hyperplasia (59/1658)
  - Increased plasma cells (plasmacytosis) (46/1658)
  - Lymphoid hyperplasia (44/1658)
  - Increased macrophages (histiocytosis) (19/1658)

  - Arteritis/periarteritis, Hemangioma (each 9/1658)
  - Lymphadenitis (4/1658)
  - Megakaryocytosis, Thrombus (each 2/1658)
  - Amyloid, Hemangiosarcoma, Lymphocytolysis (each 1/1658)

Mesenteric Lymph Node Findings

- Findings reported in five 80 week studies:
  - Sinus dilation (22/608)
  - Increased extramedullary haematopoiesis (12/608)
  - Increased plasma cells (plasmacytosis) (6/608)
  - Lymphoid hyperplasia (6/608)
  - Angiomatous hyperplasia (4/608)

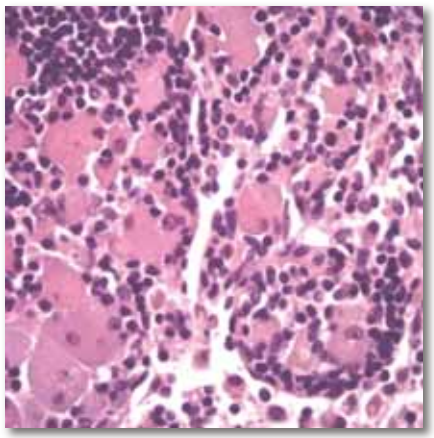
  - Hemangioma, Increased macrophages (histiocytosis) (each 1/608)

Mesenteric Lymph Node Findings

- Findings reported in fourteen 13 week studies:
  - Increased extramedullary hematopoiesis (1/294)
  - Pigment within aggregated macrophages (7/294)

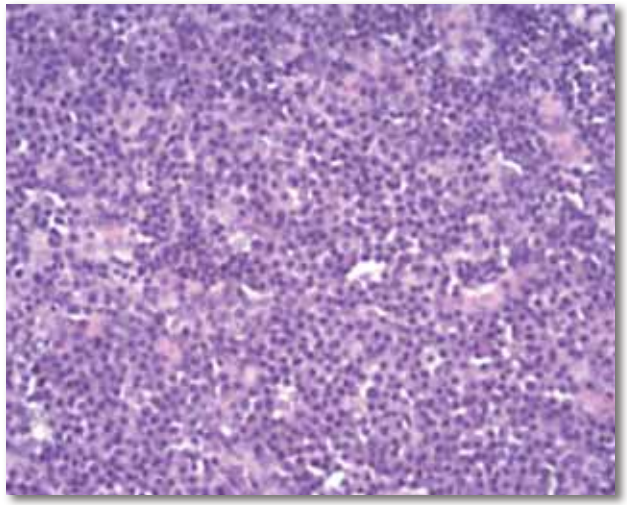
Lymph Node Findings

- Increased macrophages (histiocytosis)
  - Diffuse distension of medullary cords, or discrete foci of enlarged eosinophilic/pigmented macrophages in paracortex
  - Pigment from test item, food, haemosiderin, lipofuscin, amyloid, melanin, ceroid
  - Submandibular, mesenteric and axillary LN affected
  - May be induced by vehicles and dietary antigens e.g. gavaged corn oil may cause “microgranulomas”
  - Ageing change
  - Differential Diagnosis: Histiocytic sarcoma



Lymph Node Findings

- Increased Plasma cells (plasmacytosis)
  - Diffuse distension of medullary cords, or discrete foci of plasma cells in paracortex
  - Russell bodies evident
  - DDx Plasmacytic lymphoma infiltrates

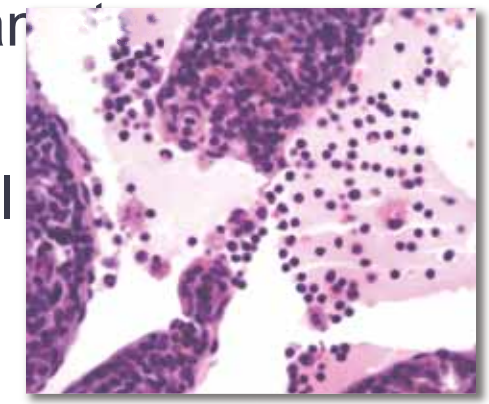


Lymph Node Findings

- Lymphoid Hyperplasia (increased lymphocytes)
  - Enlarged LN compartments and/or increased cell density
  - Follicles (B-cells) and/or paracortex (T-cells)
  - Large secondary follicles with large germinal centers
  - Result of immune stimulation eg gavage injury, i.p. dosing etc
  - T-cell expansion also seen in Peyer's patch
  - Induced in submandibular LN by sublingual bleeds, glossitis
  - Differential Diagnosis: Lymphoma

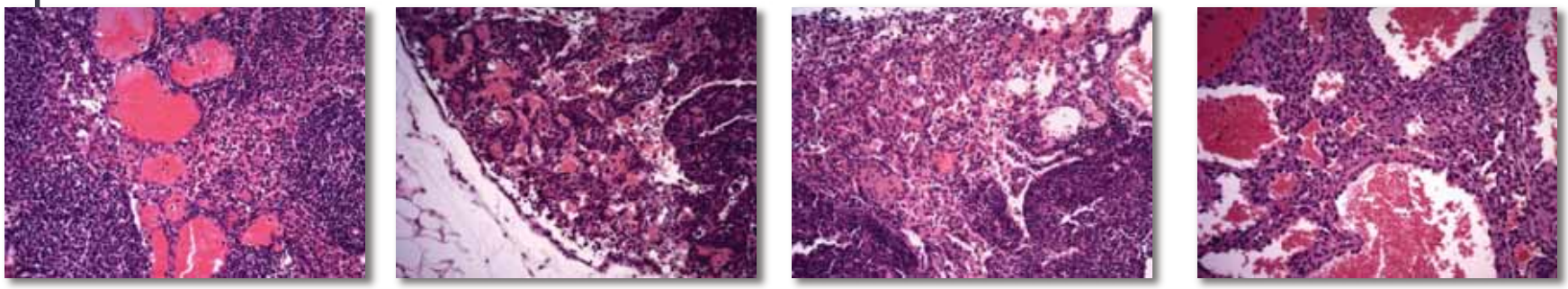
Lymph Node Findings

- Sinus Dilation
  - Focal or diffuse ageing change
  - More common in medullary sinuses
  - Sinuses dilated with eosinophilic staining material, presumably lymph with lymphocytes, macrophages, erythrocytes
  - Associated with lymphoid atrophy, because medullary sinuses expand as medullary cords decrease in diam
  - May be cystic degeneration
  - Induced in submandibular LN by sublingual bleeds



Lymph Node Findings

- Haemangiomatous/Angiomatous hyperplasia
  - Ageing change
  - Proliferation of endothelial cells
  - Continuum of changes from hyperplasia to hemangioma
  - Can be treatment related
  - Hilus can look hyperplastic in old animals so trimming plane must be taken into account



Angiectasis

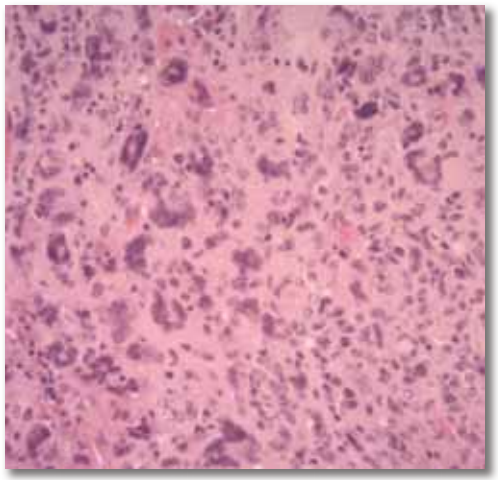
Angiomatous hyperplasia

Hemangioma

NEOPLASIA

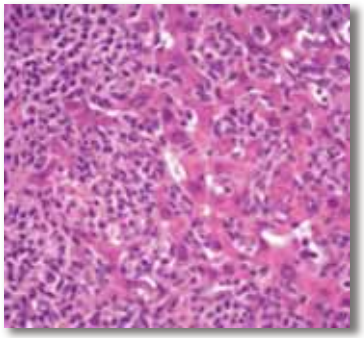
Neoplasia - Histiocytic Sarcoma

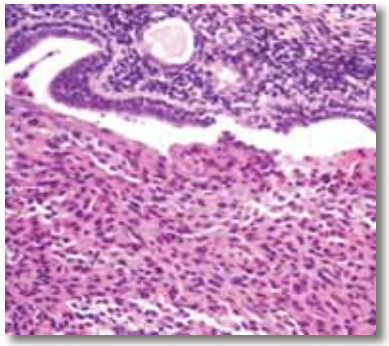
- Monomorphic or pleomorphic histocytes
- Can arise from Kupffer cells in liver as well as macrophages in Spleen, LN, Uterus
- Abundant pale eosinophilic cytoplasm
- Nucleus dark, ovoid/elongated
- Indistinct cell boundaries
- Multinucleated giant cells
- High mitotic index
- Palisading necrosis; cholesterol clefts
- Obliteration of normal architecture
- Can cause sudden death

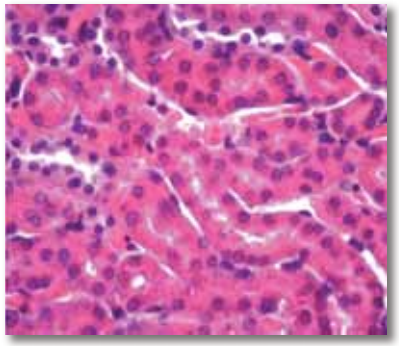


Neoplasia - Histiocytic Sarcoma

- Metastases, spread on serosal surface & vascular spaces
- Single or multiple nodular masses
- Spleen, LN, liver, lungs, uterus, subcutis
- Replace normal population of lymphocytes in spleen
- Renal PCT contains hyaline droplets caused by lysozyme degradation products from tumour cells
- 104 week studies: 56/1658 (12M, 44F)
- 80 week studies: 7/608 (1M, 6F)







Neoplasia – Lymphoma (malignant lymphoma)

- Classification of lymphomas in subtypes is not always done in routine studies in some labs, but recommended to exclude treatment related findings
- “Leukemic overspill” = neoplastic lymphocytes within blood vessel in tissue sections
- Animals found dead/too autolysed for diagnosis designated Lymphoma NOS
- Lymphoma NOS in CRL-PCS HCD:
  - 104 week studies 57/1658 (19M, 38F)
  - 80 week studies 38/608 (10M, 28F)

Neoplasia – Lymphoma

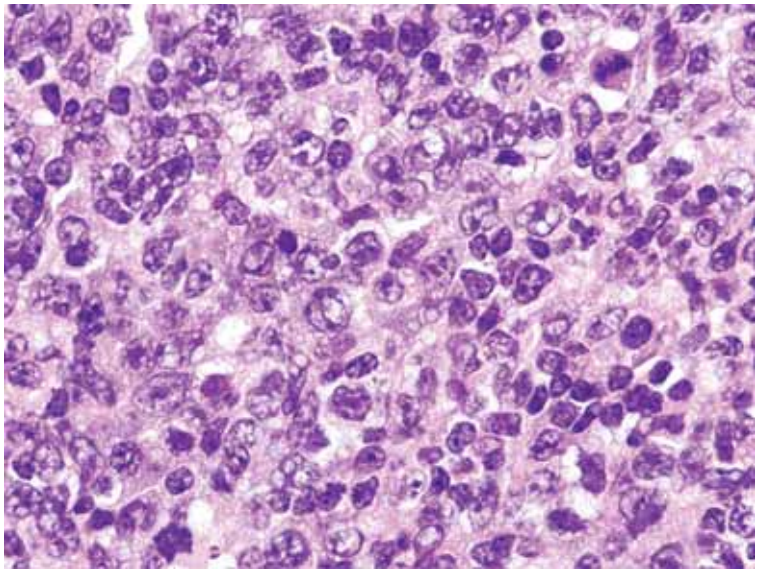
- Subtypes of lymphoma:
  - *Follicular (pleomorphic):* B cell origin but may be admixed with T helper CD4+ cells
  - *Small Lymphocyte*: B or T cell origin
  - *Lymphoblastic*: B or T cell origin, usually of T cell origin in the thymus
  - *Plasmacytic*: Origin from differentiating B lymphocytes
  - *Immunoblastic:* B or T cell origin

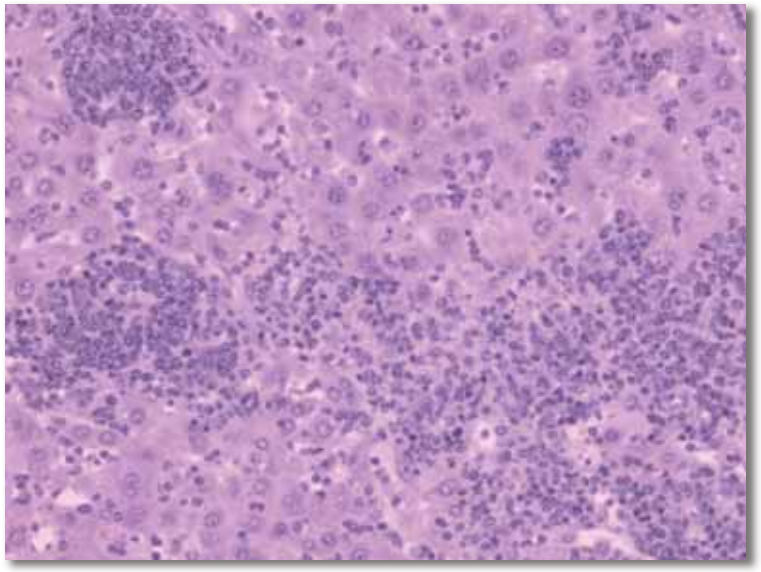
Follicular Lymphoma (Pleomorphic; FCC)

- Most common lymphoma type in CD-1 mice
- Differentiation usually B-cell; some admixed T helper CD4+
- Cell types small, medium-sized to large (or a mixture of each type) and cohesive with indistinct cell boundaries
- Moderate pale eosinophilic cytoplasm
- Large cell type (8-16 µ) has open, vesicular, cleaved/non-cleaved nucleus and high mitotic index
- Small cell type (6-10 µ) has condensed nucleus and low mitotic index
- Sometimes centrocytes, centroblasts, macrophages, immunoblasts, T-lymphocytes and plasma cells often present

Follicular Lymphoma (Pleomorphic; FCC)

- Arises in the spleen, Peyer's patches and/or mesenteric lymph node
- May form follicular pattern in spleen
  - 104 week studies 105/1658 (55M, 50F)
  - 80 week studies 9/608 (9F)



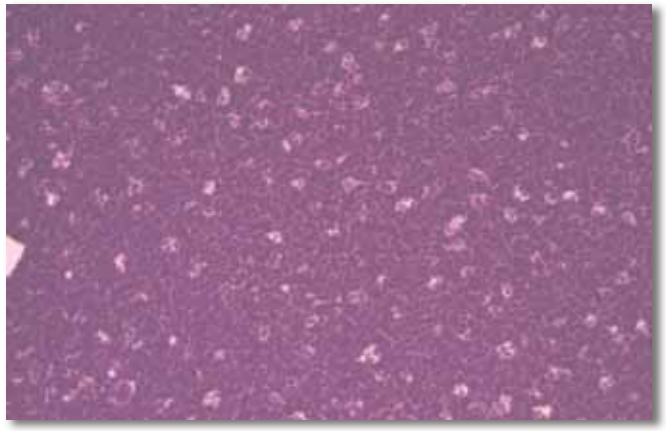


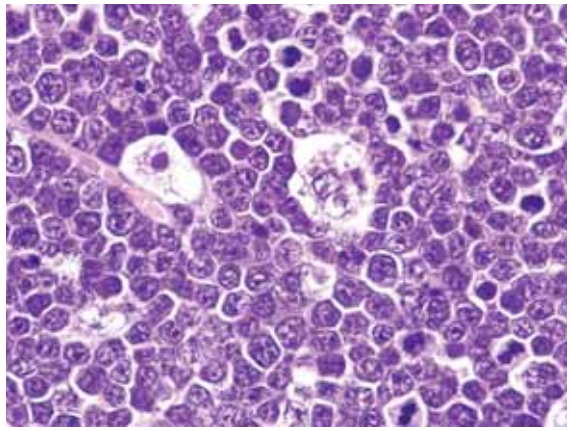
Lymphoma - Lymphocytic

- Small to medium sized lymphocyte (4-8 µ)
- Rim of scant pale basophilic cytoplasm
- Condensed nucleus with stippled chromatin pattern
- Uniform sheets, non-cohesive; Low mitotic index
- Starts in Thymus or LN, spreads to spleen and other organs obliterating the architecture
- Can cause activation of histiocytes
- May get collision tumour with histiocytic sarcoma
- Occurs after 6 month of age
- 104 week studies 97/1658 (38M, 59F)
- 80 week studies 1/608 (1M)

Lymphoma - Lymphoblastic

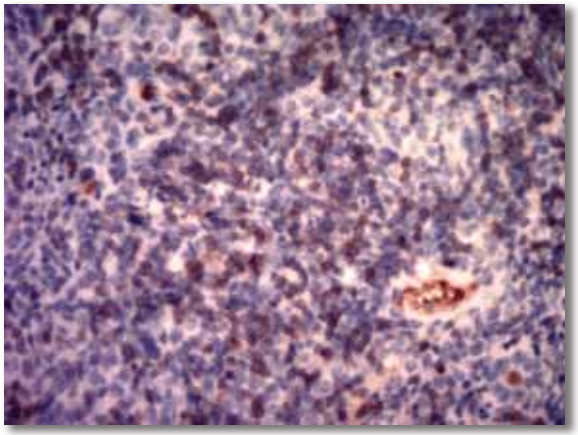
- Lymphoblast (7-12 µ) ; differentiation T or B
- Moderate to scant pale basophilic cytoplasm
- Immature nucleus with fine stippled “sprayed on” chromatin
- Round nucleus; high mitotic index
- Uniform sheets – non-cohesive (“boiling peas” appearance)
- Tingible body macrophages common (single cell necrosis and apoptosis) - “starry sky” appearance

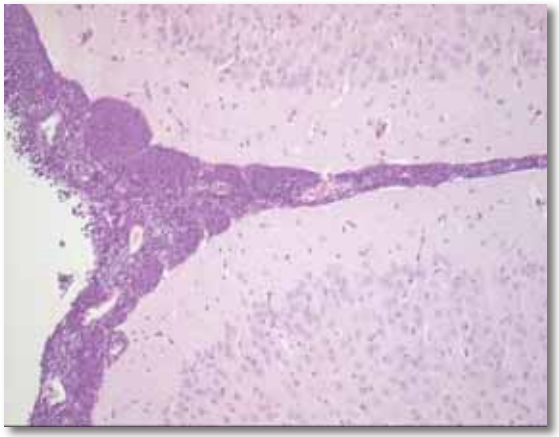




Lymphoma - Lymphoblastic

- Starts in Thymus or BM; spreads to spleen, LN, liver, lungs, CNS, kidney, GIT, genito-urinary tract, leukemic overspill
- Increased incidence with age; recorded on subchronic studies
- Often recorded in “unscheduled sacrifice” animals
- 104 week studies 21/1658 (7M, 14F)
- 80 week studies 5/608 (5M)
- Usually CD3+





Lymphoma - Plasmacytic

- Very rare as Spontaneous tumours
- Mature Plasma cells (7-16 μ)
- Abundant pale amphophilic cytoplasm; Russell bodies
- Nucleus round, eccentric, condensed clock face chromatin
- Non-cohesive; variable mitotic index
- May be induced by i.p. injections if vehicle is mineral oil
- Starts in draining LN causing obliteration of normal architecture

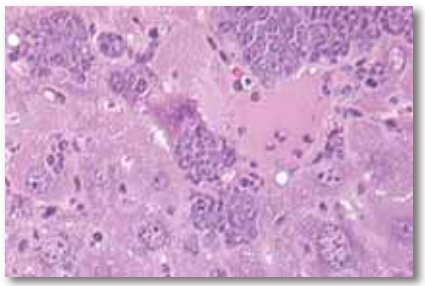
- 104 week studies 1/1658 (1F)
- 80 week studies - not recorded

Lymphoma - Immunoblastic

- Very Rare !
- Large cells (10-18 μ) – B or T
- Moderate pale eosinophilic/amphophilic cytoplasm
- Nucleus round, vesicular, eccentric with prominent nucleolus
- Non-cohesive sheets
- High mitotic index - very aggressive with sarcomatous involvement of extra-lymphoid sites e.g. pancreas
- 104 week studies 2/1658 (1M, 1F)
- 80 week studies 2/1658 (2F)

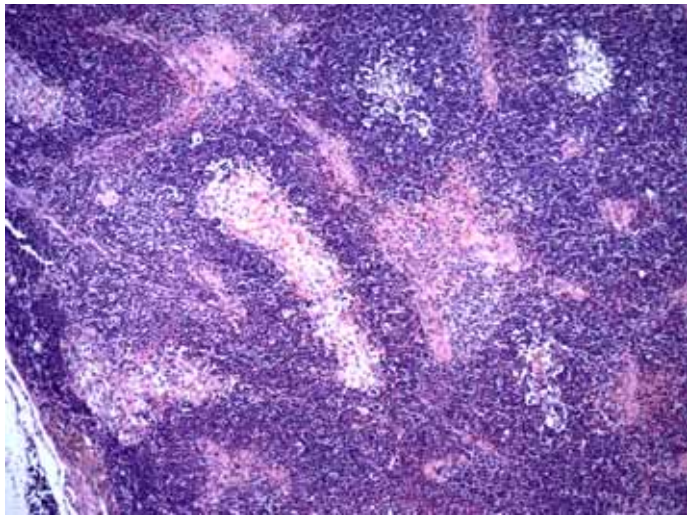
Leukemia, granulocytic

- Immature myeloid cells (usually neutrophilic)
- Large cells, pale staining cytoplasm, notched nuclei, cytoplasmic granules
- Arises in splenic red pulp and spreads to liver, kidney, lungs, CNS (see greenish tinge at necropsy)
- Neoplastic cells present diffusely in BM, and vascular spaces
- Mononuclear leucocytosis on hemogram
- 104 week studies 13/1658 (10M, 3F)
- 80 week studies 5/608 (1M, 4F)
- Differential Diagnosis: Lymphoma, EMH
  - EMH should have erythroid elements
  - Leukemia much rarer than lymphoma



Thymoma

- Neoplastic epithelial components, with or without neoplastic lymphocytes, centrally located within lobules
- Well encapsulated
- Moderate mitotic index, though benign
- Various levels of differentiation
- Relatively rare
- 104 week studies 3/1658 (1M, 2F)
- 80 week studies 4/608 (3M, 1F)



Summary

- Most Common findings in Crl:CD-1 mice
  - Hyperplasia (thymus, spleen, lymph node)
  - Lymphoid depletion (thymus)
  - Arteritis/periarteritis (thymus)
  - Sinus dilation (lymph node)
  - Increased EMH (spleen, lymph node)

- Lesions of Hematopoietic system uncommon in 13 week studies, but high incidence in carcinogenicity studies (80 or 104 week)
- Most common cause of death in carcinogenicity studies is lymphoma

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