Differentiation of Hematopoietic and Immune System Reactive Lesions (Hyperplasias) from Neoplasias Using Anatomical and Immunohistochemical Considerations

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Today’s Presentation

• Lymph Nodes
• Spleen
• Thymus

• Reactive lesion = any responses of a tissue or cell to any type of insult or condition, or from physiological changes; may be a hyperplastic lesion
Descriptive vs Interpretive terminology

• Enhanced Histopathology of The Immune System (Susan Elmore)
  *Descriptive rather than interpretive terminology should be used to characterize changes*

• Routine Diagnostic Histopathology (Jerry Ward and Alys Bradley)
  *Interpretative terminology is used*
Formalin-fixed, Paraffin-embedded (FFPE)

Immunohistochemistry: Mouse

- B cells – CD45R, Pax-5, CD79a
- Immunoglobulins – human kappa light chains, IgM, other Iggs
- T cells – CD3, Tdt, CD134
- Histiocytes – lysozyme, Mac-2, F4/80, CD68
- Granulocytes – MPO, ym1/2(Ch1I3), iNOS, CD43, CD45

References
Kunder S et al, Toxicol Pathol 35: 366, 2007;
Ward JM et al, Tox. Pathol. 34: 616, 2006; Rehg J et al, Vet Path 2012
Mikaelian I et al, Tox Path 32:181, 2004,

Mouse Lymph Node
Anatomical Compartments:
Keys To Understanding Lymphoid Tissues

- B follicles
- Cortex
- T-cells
- PC Paracortex
- Medulla
- Hilus
Mouse LN Sections – plane of section

what is normal & diagnosis
Monkey and Human Lymph Nodes

prominent germinal centers
LN Reactive Lesions


Fig. 1.—Simplified schematic representation of reactive lesions in rodent lymph nodes. C = cortex; P = paracortex; M = Medulla.
Lymph Node “Hyperplasia”

Enlarged LN

- An increase in number of normal cells in a tissue or organ, excluding tumor formation, whereby the bulk of the part or organ may be increased.
Plasma Cell Hyperplasia
Immunoglobulin (human kappa light chains) expressed in plasma cells

Plasma cells in lymph node
Mouse LN

Germinal Center

Plasma cells
Germinal Centers Vs Lymphoma

GC  Lymphoma  Lymphoma
Normal Mouse Spleen

The mouse spleen often serves as bone marrow in responses to conditions
Mouse Spleen

White pulp

Germinal Center

Red pulp

T-cell zone = PALS
periarteriolar lymphoid sheath
Splenic White Pulp Lesions


NORMAL
T CELL DEPLETION
B CELL DEPLETION
ATROPHY
FOLLICULAR HYPERPLASIA
FCC LYMPHOMA

B = B-cell zone
T = T-cell zone, PALS
Mouse 5 Gram Spleen
Mouse Malarial Spleen Immune Responses

Normal spleen

Malaria 10-14 days

Plasma cells

Igs
Reactive spleen
Ki67
Lymphoid & Erythroid Hyperplasia

Ki67 Normal Spleen
Tox and Carcinogenesis Studies
“Lymphoid Hyperplasia”
Splenic Red Pulp Lesions

• Myeloid
  Granulopoiesis
  Myeloid leukemia

• Histiocytosis
  Histiocytic sarcoma

• Erythroid hyperplasia
  Erythroblastosis
  Erythroleukemia

• Plasma cell hyperplasia

• Megakaryocytic hyperplasia
  Megakaryocytic leukemia

• Combinations of all of the above
<table>
<thead>
<tr>
<th>Myeloid hyperplasia</th>
<th>Granulocytic leukemia</th>
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<tbody>
<tr>
<td>All stages of granulocyte development present</td>
<td>Usually immature cells with one stage predominating</td>
</tr>
<tr>
<td>Erythropoietic activity usually present</td>
<td>Erythropoietic activity absent</td>
</tr>
<tr>
<td>Megakaryocytes are usually numerous</td>
<td>Megakaryocytes are few and present in organs where they are normally found</td>
</tr>
<tr>
<td>Frequently associated with inflammation</td>
<td>Not usually accompanied by inflammation</td>
</tr>
<tr>
<td>Cells are not invasive</td>
<td>Cells are invasive</td>
</tr>
<tr>
<td>Normal tissue of the spleen is relatively unaffected</td>
<td>Normal tissue of the spleen is extensively replaced</td>
</tr>
<tr>
<td>Blood is normal or leukocytosis with mature forms</td>
<td>Blood usually contains immature forms and may have high white blood count</td>
</tr>
<tr>
<td>Hemorrhages are absent</td>
<td>Hemorrhages are frequent</td>
</tr>
<tr>
<td>Not transmissible</td>
<td>Transmissible</td>
</tr>
<tr>
<td>Not transplantable</td>
<td>Transplantable</td>
</tr>
<tr>
<td>Can occur at any age</td>
<td>Usually occurs in mice over 12 months old</td>
</tr>
<tr>
<td>When the lymph nodes are involved the cells are usually confined to the medulla</td>
<td>When the lymph node is involved the cells are not confined to the medulla</td>
</tr>
<tr>
<td>Bone marrow is involved late</td>
<td>Bone marrow is involved early</td>
</tr>
<tr>
<td>Bone marrow involvement does not result in destruction of the bone</td>
<td>Bone marrow involvement results in destruction of the bone</td>
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Spleen

“Hematopoietic Cell Proliferation”
Erythroid and Myeloid Hyperplasia

Normal Mouse Spleen

Hyperplasia
Splenic Myeloid and Erythroid Hyperplasia
Splenic Myeloid Hyperplasia

Myeloperoxidase

κ light chains
Mouse Spleen - Erythroid Hyperplasia
Splenic Erythroid Hyperplasia
Myeloid Hyperplasia vs Myeloid Leukemia

Hyperplasia

Leukemia
Large Rat Spleen
Early Lymphomas vs Lymphoid Hyperplasia
Early Lymphoma, Follicular in A B6C3F1 Mouse
Mesenteric LN  B6,129
Lymphoma
Early, Focal
(atypical hyperplasia)

B6C3F1
Follicular Lymphoma

Normal

Early Lymphoma
CD45R Expression
Normal B Cells vs Lymphoma Cells
Mouse Lymphoproliferative Disorders (LPD)

- SJL/J
- MAIDS
- Lpr/gld
- Gamma Herpes virus induced lpd (MHV68)
- Others
Mutant Mice
Heritable Lymphoproliferative Diseases
*Gld or lpr* mutants

Generalized lymphoproliferative (gld) disease
5 months old

resembles small lymphocyte lymphoma composed of T cells CD3+/CD45R+
defect in ability to undergo antigen stimulated apoptosis
Heritable
Autoimmune Lymphoproliferative Syndromes in Humans and Mice

- Human genetic disorder – FAS, FASLG, mutations > LP disorders, ALPS syndrome and lymphoma
- Mice – spontaneous autosomal recessive mutations in mice, autoimmune disorder, GN, LP syndrome (Lpr, Gld)
- Mouse Lpr – Fas (lpr; APO1; APT1; CD95; TNFR6; Tnfrsf6; A1196731); WF Davidson et al, J Immunol 133:1048, 1984
- Mouse Gld – Fasl (gld; CD178; CD95L; Fas-L; Faslg; CD95-L; Tnfsf6; APT1LG1; Fas-Ligand) 40kd type II membrane protein (WF Davidson et al, Proc Natl Acad Sci USA 82:1219, 1985; WF Davidson et al, J Exp Med 187:1825, 1998)
- Mice – LP disease, large nodes and spleen, resembles small lymphocyte lymphoma, T cells, CD45R+/CD3+, defect in ability to undergo antigen stimulated apoptosis
- Mice – aging > non-clonal lymphoproliferative disorder > clonal B cell lymphomas (follicular lymphomas)
Proof of Neoplasia

• **Invasion & Metastases**

• **Transplantation**

• **Immunophenotype (IHC, FACS)**

• **Genetics (including clonality assays)**
  
  **Gene rearrangements (T an B lymphocyte)**
  

  **Kappa or lambda light chains**

  Spectral karyotyping – CF Qi et al, J Pathol 221: 106, 2010

  **Comparative genomic hybridization –**

  Yamaguchi et al, Mutat Res/Fund Mol Mech Mut 686: 30, 2010

  **Immunoglobulin and TCR sequencing -** CF Qi et al, J Pathol 221: 106, 2010

  **Gene expression profiling -** CF Qi et al, J Pathol 221: 106, 2010

High-molecular-weight DNAs were prepared from lymphoid tissues, digested with restriction endonucleases, separated by electrophoresis in 0.7% agarose gels, transferred to nitrocellulose, hybridized with 32P-labeled probes by established techniques. Detection of immunoglobulin heavy chain (IgH) rearrangements used EcoRI and the J11 JH probe; for TCRb rearrangements, HpaI and the Tb probe were used.
Thymus
Histopathogenesis of Thymic T-cell Lymphomas chemicals, GEM, retroviruses, irradiation
Thymic Atypical Medullary Hyperplasia

In p53 +/- and -/- mice, virally and chemically induced lymphomas

JK Dunnick, Hardisty, Herbert, Seely et al, Tox Pathol 25: 533, 1997
JM Ward et al, Lab Invest 79: 3, 1999
Rat Chemically-induced Early Thymic Lymphoma
Thymus – Aging Mice  Nodular Hyperplasia, B-cell

B6,129

CD45R
Other Tissues
What Is Your Diagnosis?
Rat *Mycoplasma sp* infection
Lymphoma Diagnosis Prevention Pathology Program

*Dissect Out The Lesion From Top to Bottom*

- Observe low magnification anatomy/histology of thymus, spleen or lymph node
- Compare cytology of normal areas (white pulp of spleen, cortex or paracortex of node) with possible abnormal areas
- Is there enough cytological change to diagnose lymphoma?
- Are other tissues involved?
- Is there a cause of reactive lymphoid tissue?
- Use clonality assays and other techniques
- Integrate H&E, IHC and other data - including mechanism (s) involved
- Make a final diagnosis (if in doubt, use “atypical hyperplasia” or enhanced immune system histopathology terminology)
If In Doubt

• Show the slides around
  (to colleagues, email to other tissue experts)

• Pathology peer review