Fundamental Concepts to Understand Adverse Effects in the Immune/Lymphoid System

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Outline of the Presentation

1. Introduction
2. Ontogeny and components of the lymphoid system
3. Evaluation of the lymphoid system
4. Immunotoxicity
5. Stress and the lymphoid system
6. The lymphoid system and juvenile studies.
7. Summary and conclusions

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1. Introduction

- The **lymphoid system** consists of primary **lymphoid** organs, secondary **lymphoid** organs, and lymphatic vessels
- Associated organs that compose the **lymphoid tissue** are the sites of lymphocyte production.
- The **lymphoid system** enables lymphocytes to encounter antigens and it is here that **adaptive immune responses** are initiated.
- The bone marrow produces blood cells that are involved in **innate immune responses**.

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1. Introduction
What is the importance of detecting adverse effects in the Lymphoid System?

- Regulatory agencies (US and EC) - immunotoxicity testing should be performed on all new investigational drugs and medicinal products.

- Gross and histopathological examination of lymphoid tissues are necessary and pivotal steps in the assessment of new drugs for immunotoxic potential.

Haley et al, Toxicologic Pathology,33: 404-407, 2005

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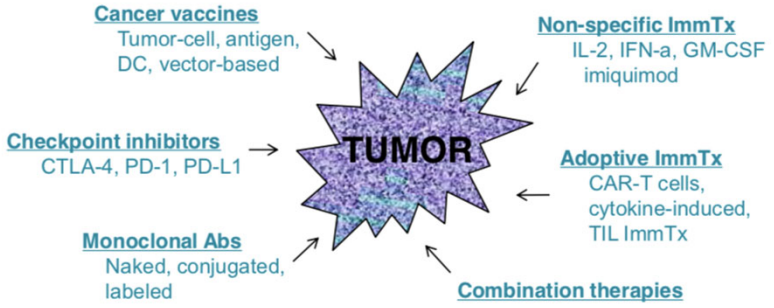
Types of Immune-modulating Therapies

- Historical pharmacologic agents (e.g. steroids)
- Vaccines
- Hyposensitization/tolerance (e.g. allergy shots)
- Cancer immunotherapies



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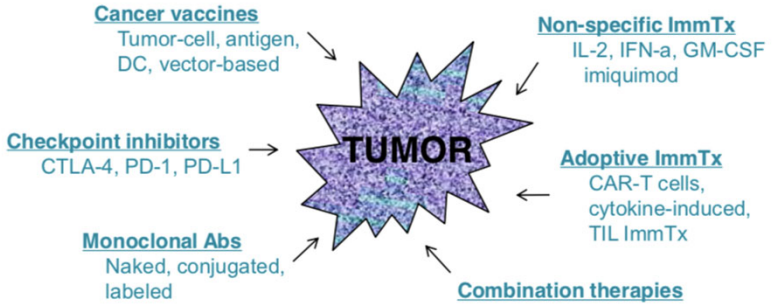

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2. Ontogeny and Components of the Lymphoid System

- Bone marrow and blood
- Bursa of Fabricius (birds) or Bursa equivalent organ (others)
- Thymus
- Spleen
- Lymph nodes
- MALT – mucosa associated lymphoid tissues
- BALT – bronchus associated lymphoid tissue

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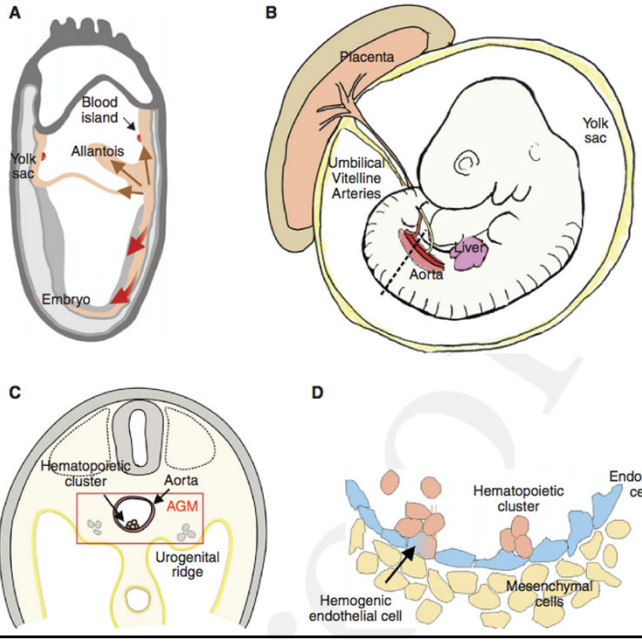
Ontogeny of the Lymphoid System



AGM : aorta-gonad-mesonephros
The **aorta-gonad-mesonephros** (**AGM**) is a region of embryonic mesoderm that develops during embryonic development from the para-aortic splanchnopleura in chick, mouse and human embryos. It has been suggested that this area, in particular the ventral wall of the dorsal aorta, is one of the primary origins of the definitive haematopoietic stem cell.

Dzierzak & Philipsen,
Cold Spring Harbor Perspectives in Medicine,
2013 (open access)

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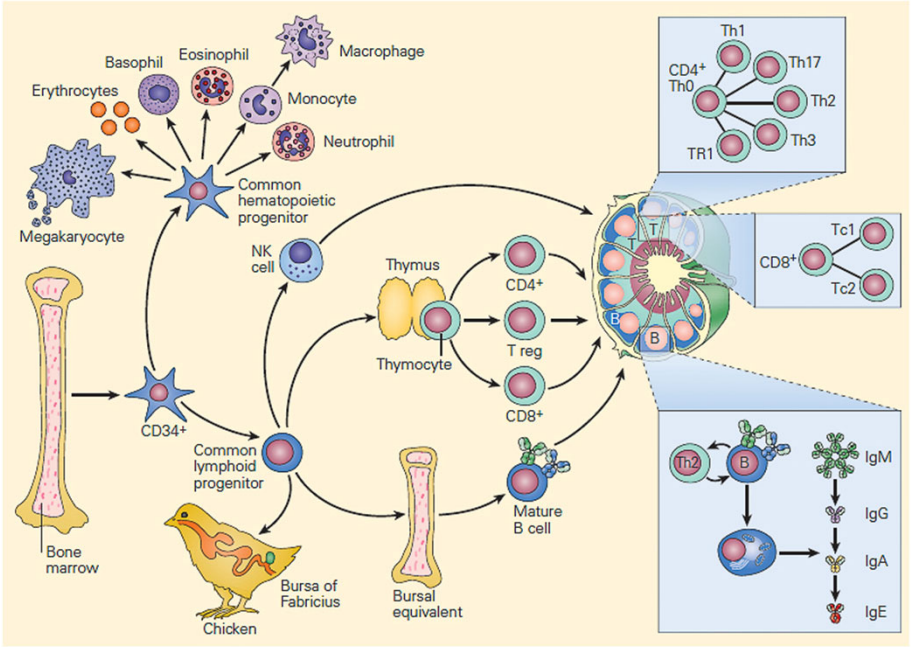
Ontogeny of the Lymphoid System



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Components of the Lymphoid System

- Bone marrow (**primary**)
- Bursa of Fabricius (birds)/Bursa equivalent organ (others) (**primary**)
- Thymus (**primary**)
- Spleen (**secondary**)
- Lymph nodes (**secondary**)
- MALT (**secondary**)
- BALT (**secondary**)

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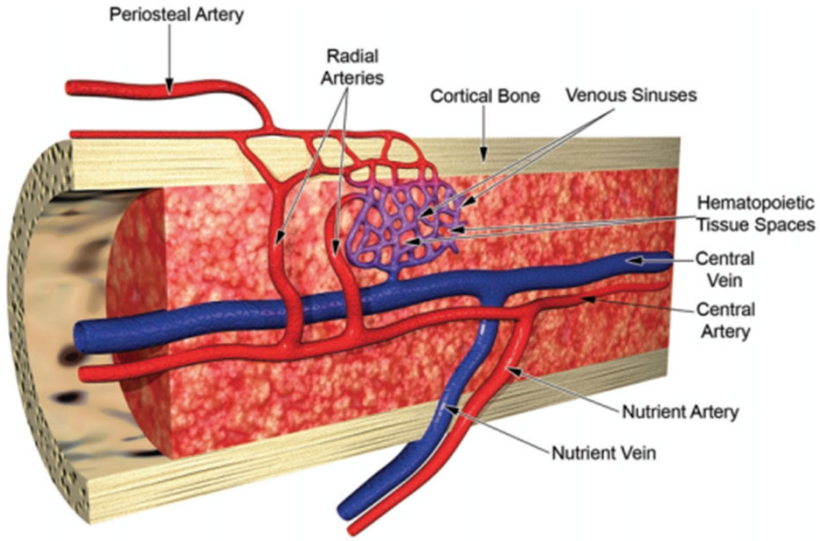
Histology of the Bone Marrow



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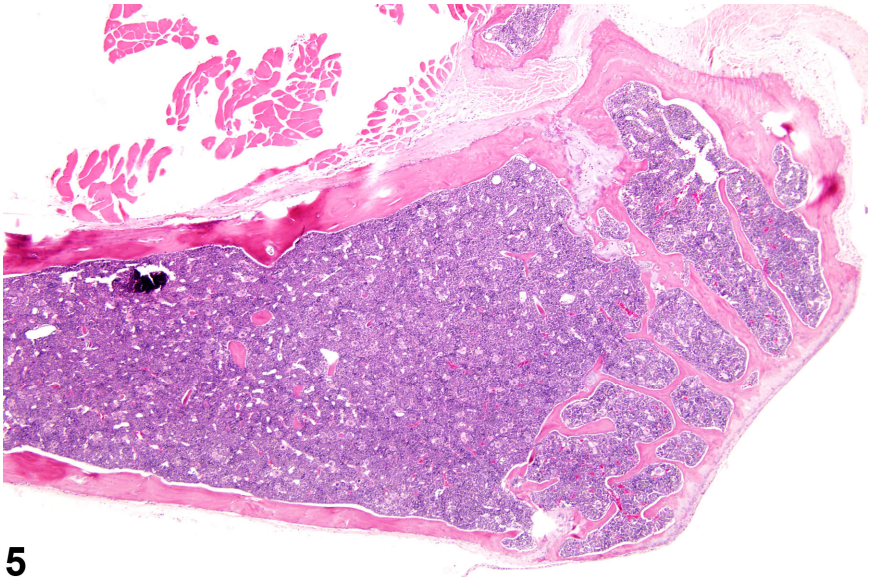
Histology of the Bone Marrow



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From: https://ntp.niehs.nih.gov/nnl/hematopoietic/index.htm

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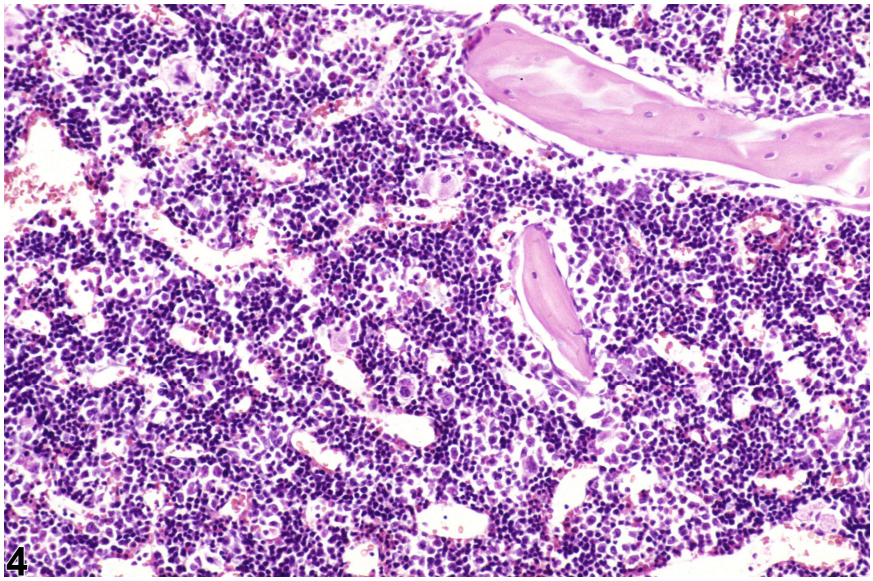
Histology of the Bone Marrow



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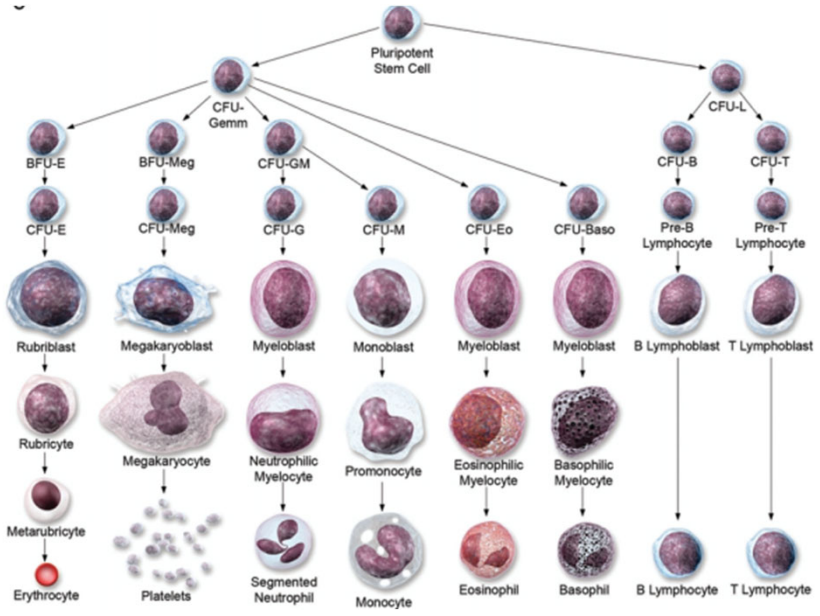
Histology of the Bone Marrow



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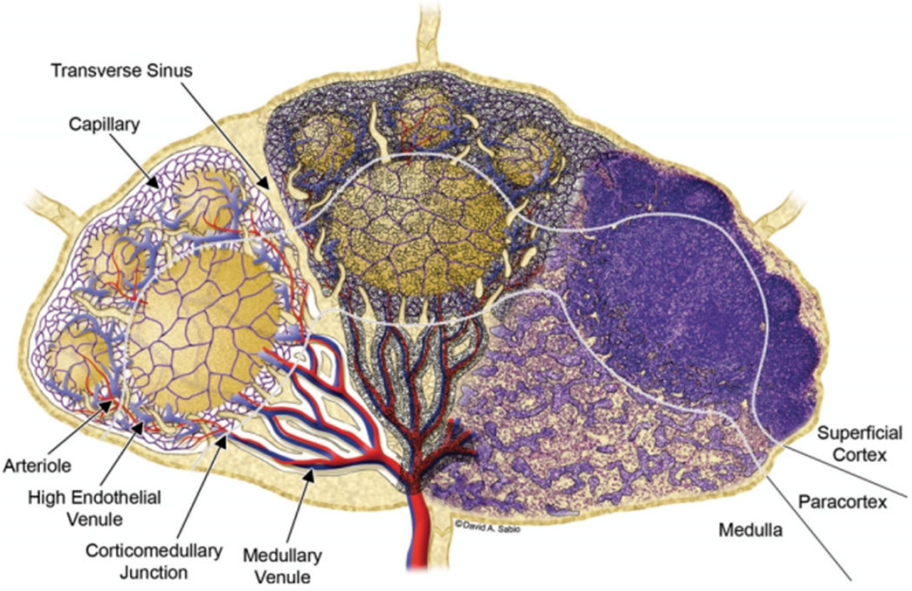
Histology of the Lymph node



Willard-Mack Tox Path 34(5), 2006

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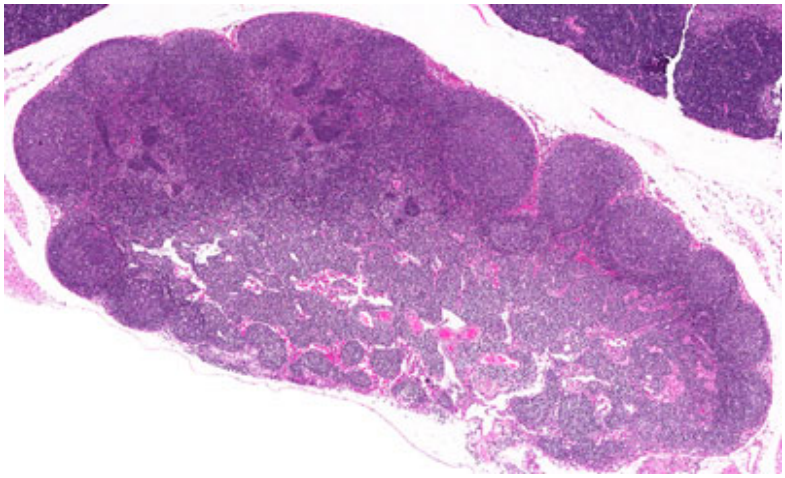
Histology of the Lymph node



From: https://ntp.niehs.nih.gov/nnl/immune/index.htm

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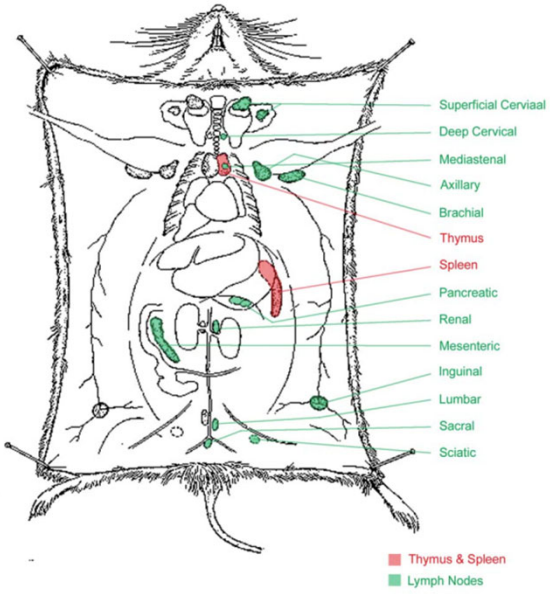
Distribution of Lymph nodes in the mouse



Mouse image from: The virtual mouse necropsy. (http://www.geocities.com/virtualbiology/necropsy.html)

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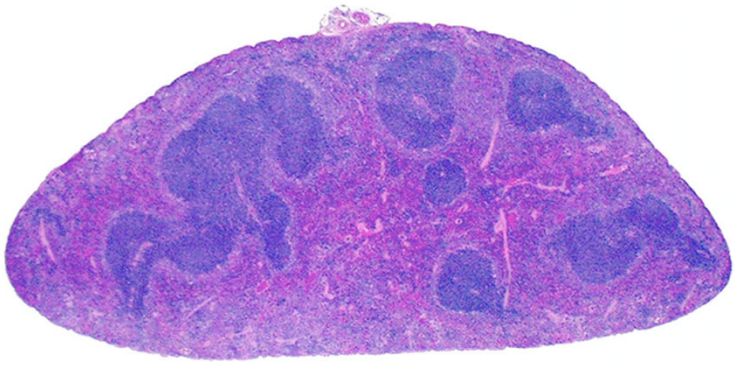
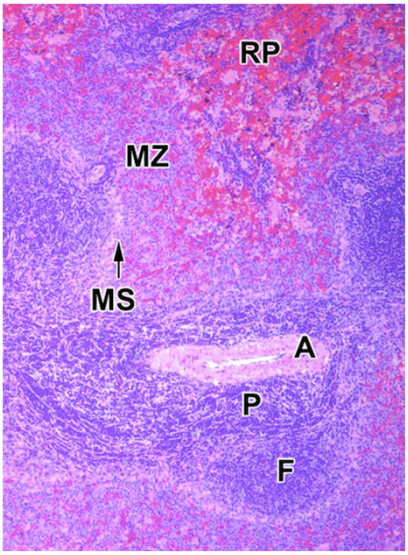
Histology of the Spleen





RP= red pulp, A= artery, MZ= marginal zone, MS= marginal sinus, P= PALS, F= follicle, P= periarteriolar sheath

ImageS from a CD provided with Toxicologic Pathology Volume 34, No. 5, 2006

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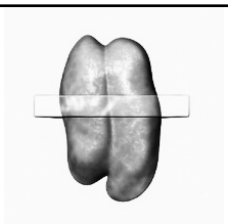
Histology of the Thymus



Rat thymus

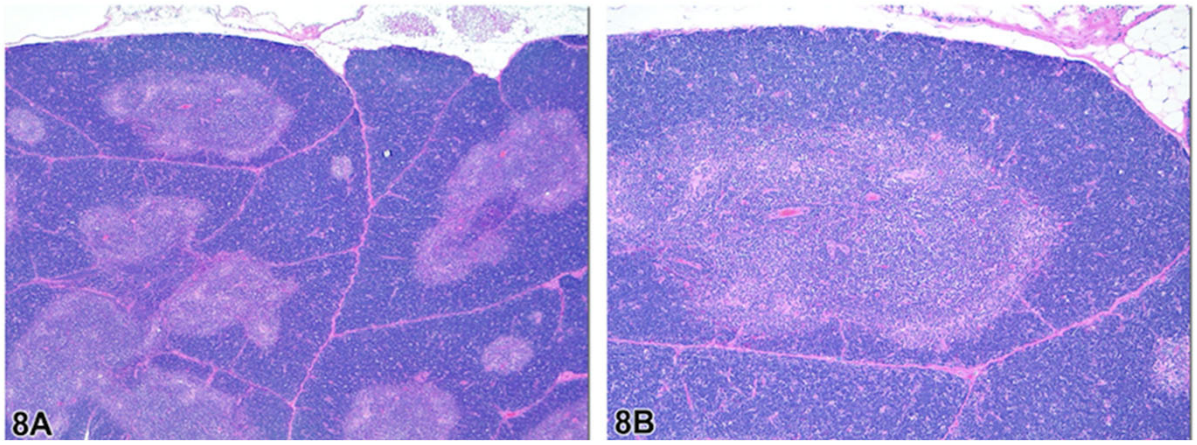


Image from a CD provided with Toxicologic Pathology Volume 34, No. 5, 2006

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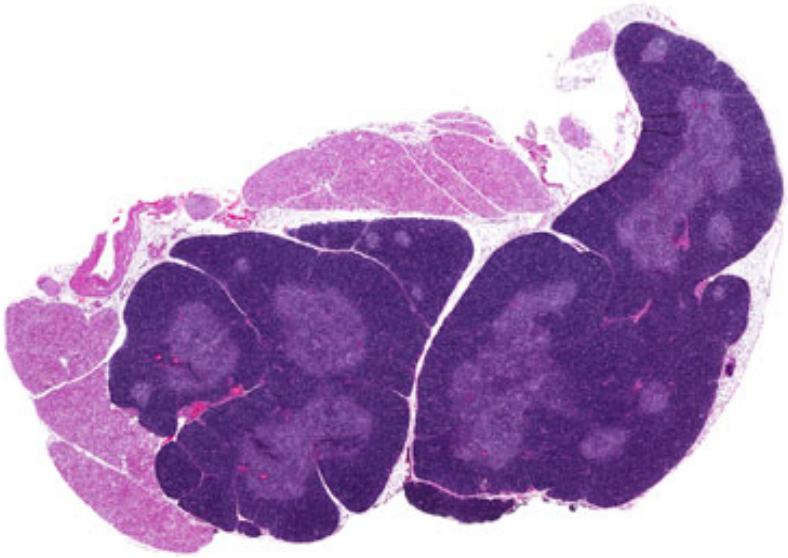
Histology of the Thymus



https://ntp.niehs.nih.gov/nnl/immune/thymus/index.htm

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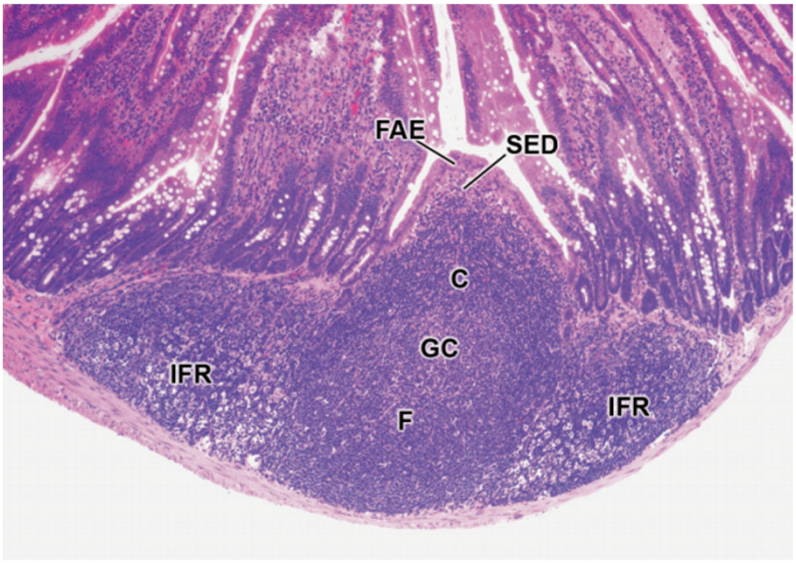
MALT – Mucosa associated lymphoid tissue



Cesta MF **Normal Structure, Function, and Histology of Mucosa-Associated Lymphoid Tissue. Tox Path** Volume 34 Issue 5, August 2006

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3. Evaluation of the Lymphoid System

- Clinical pathology
- Lymphoid organ weights
- Histopathology - **compartments**!

Haley et al, Toxicologic Pathology,33: 404-407, 2005

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Evaluation of the Lymphoid System

a. Is the lymphoid organ macroscopically increased or decreased in size?

b. Which compartment is specifically involved?

c. Is the change in size of the organ due to a change in components (e.g., cells, stroma, edema fluid) of a particular compartment?

d. Is this change in size due to a change in cell numbers in one or more compartments, i.e., microscopically increased or decreased number of cells, and if so which cells are involved ?

Haley et al, Toxicologic Pathology,33: 404-407, 2005

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Evaluation of the Lymphoid System Enhanced histopathology approach

- Enhanced histopathology approach is based on overall evaluation of lymphoid organs/tissue and their compartments.
- It may involve semi quantitative analysis of the B, T and other compartments (cellular constituents, alterations in numbers, location, composition).
- Enhanced histopathology approach may use immunohistochemistry to detect specific lymphoid cell types

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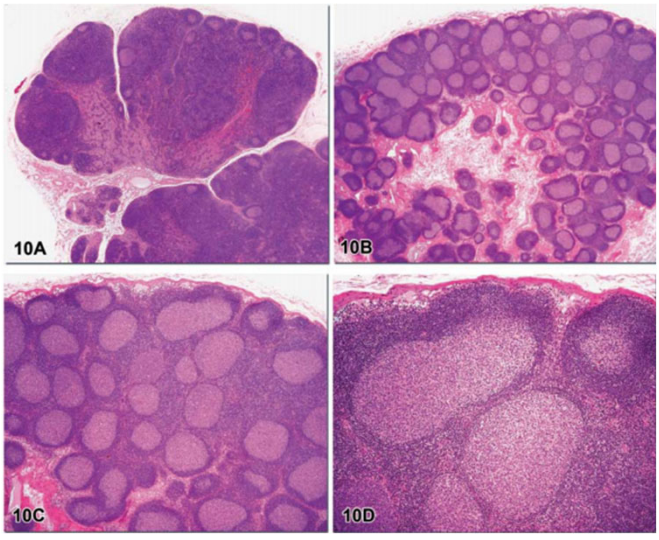
Evaluation of the Lymphoid System Enhanced histopathology approach



Elmore - Tox Path 34: 634-647, 2006

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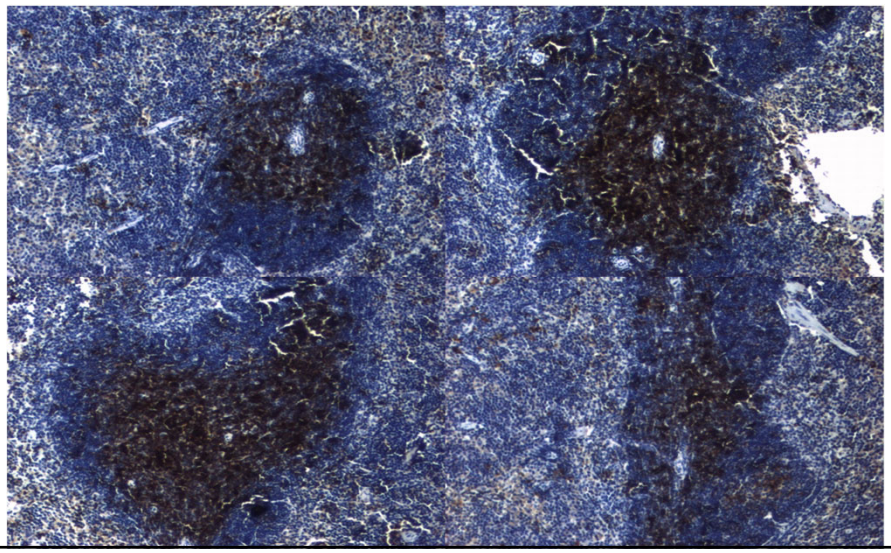
Evaluation of the Lymphoid System Enhanced histopathology approach

CD3+ cells



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Evaluation of the Lymphoid System Enhanced histopathology approach example

- Checklist for histopathological evaluation of the **thymus**
  - Cortex
  - Medulla
  - Cortex/medula ratio
  - Epithelium free areas (EFAs)
  - Other
    - Inflammation
    - Cysts
    - Pigments
    - Extramedullary hematopoiesis

- Obs. Answers must include information on:
  - increases/decreases
  - the severity/grades

Elmore SA 2006. Enhanced histopathology of the thymus Tox Path 34:656-665

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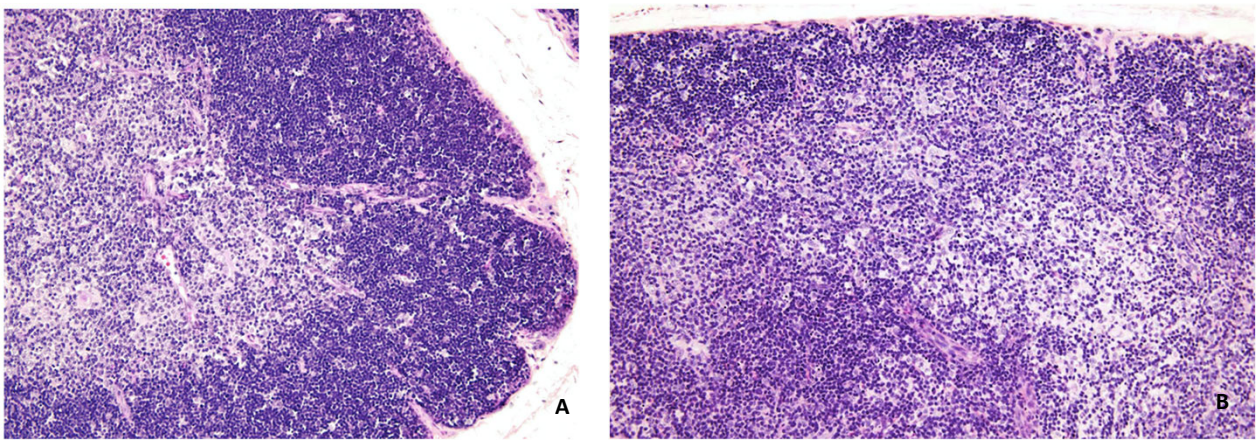
Evaluation of the Lymphoid System
Enhanced histopathology approach example



Sprague-Dawley rat thymus, treatment with Dioxin
A – control; B- treated

Elmore SA 2006. Enhanced histopathology of the thymus Tox Path 34:656-665

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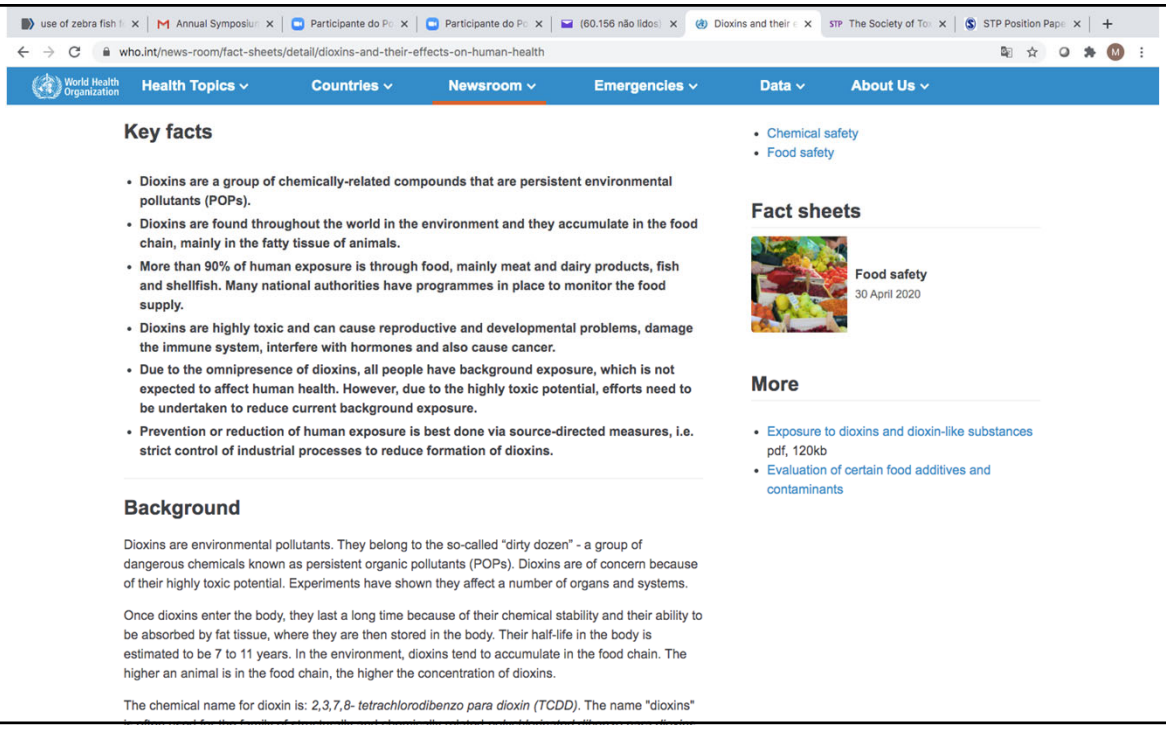

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4. Immunotoxicity

- Definition:
- "any adverse effect on the components of and/or function of the immune system by a biological, chemical, or physical agent resulting from either direct or indirect actions and reflecting either permanent or reversible toxicity."

Hinton, Tox Path 28 (3) 467-478, 2000

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Immunotoxicity – adverse effects
Five Areas of adverse effect categories defined by FDA for the immunotoxicology evaluation of new drugs

a. **Immunosuppression**: Effects on the immune system that result in decreased immune function

b. **Immunogenicity**: Immune reactions elicited by a drug and/or its metabolites

c. **Hypersensitivity**: Immunological sensitization due to a drug and/or its metabolites

d. **Autoimmunity**: Immune reactions to self-antigens

e. **Adverse Immunostimulation**: Activation of immune system effector mechanisms

FDA 2002 – Guidance document.

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How to characterize immunotoxicity?

- Two levels of testing are defined:
- **Level I** tests do not require further perturbation of the test animal (eg, by injection of test antigen) and can be done with the same animals used in a standard toxicity study (acute, subchronic, and reproduction).
- **Level II** tests are defined as functional tests and usually require a concurrent satellite group of test animals or an additional follow-up study to evaluate immunologic function.

Hinton, Tox Path 28 (3) 467-478, 2000

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How to characterize immunotoxicity?

- Hematology – including differential cell counting
- Total serum immunoglobulin level
- Weights of lymphoid organs (thymus, spleen, lymph nodes, mesenteric and popliteal)
- Histopathology of lymphoid tissues (thymus, spleen, lymph nodes, mucosa associated lymphoid tissue)
- Immunohistochemistry of lymphoid tissues
- Flow cytometry of lymphoid suspensions

Van Loveren et al., Therap Innov and Reg Sci, 1996

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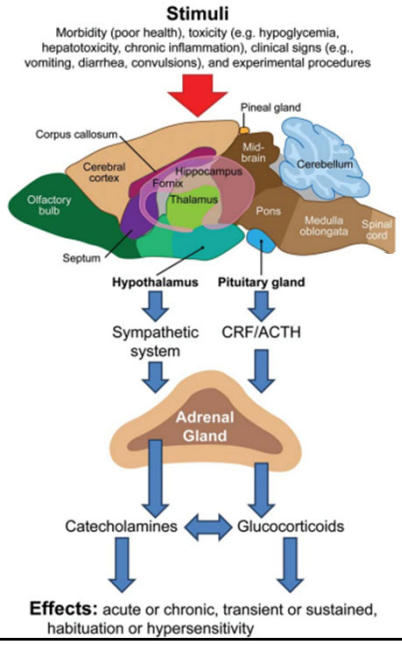
Stress and the Lymphoid System



Systems affected: In-life parameters, hematology, organ weights, immune system, endocrine, reproductive, neural, clinical signs

Everds et al., ToxPath 41: 560-614, 2013

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Stress and the Lymphoid System

- Glucocorticoids induce the **apoptosis of lymphocytes** and alter leukocyte migration and redistribution;
- A major component of their action is the **inhibition of cytokines**, resulting in a decreased release of interleukins (IL), interferons (IFN) and tumor necrosis factor (TNF), such as IL-2, IL-6, IFN-γ and TNF-α.

Meier, CA, https://doi.org/10.1530/eje.0.1340050

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Stress and the Lymphoid System

- Stress effects on organ weights :
  - Thymus weight decreased
  - Spleen weight decreased
- Stress induced alterations in histology :
  - Thymic cellularity decreased
  - Spleen cellularity decreased.

Everds et al., ToxPath 41: 560-614, 2013

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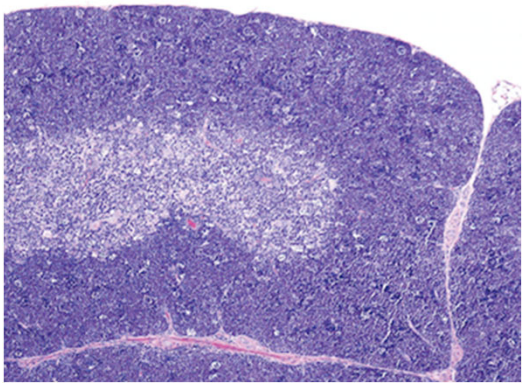
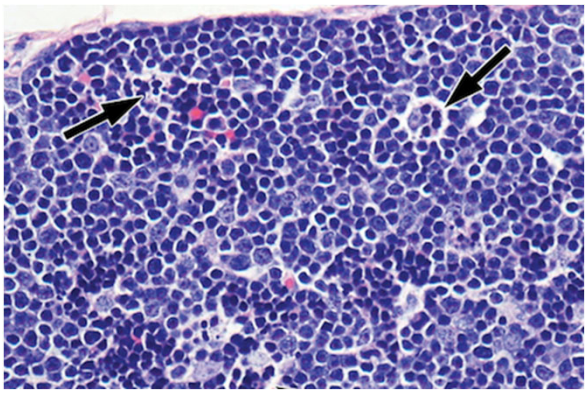
Stress and the Thymus





Pearse, Tox Path 34(5), 2006

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Importance of Lymphoid System in juvenile studies

- A number of regulatory guidelines have emerged to address the safety of drugs intended for pediatric indications.
- Developmental immunotoxicity (DIT) testing - exposure to immunotoxicants early in development may result in **enhanced susceptibility of, or unique or more persistent effects on, the immune system, in comparison to adult exposure.**
- The best approach to DIT is to address the possible impacts of exposure during all of the critical windows of development

HOLSAPPLE AND O'LONE, Toxicologic Pathology, 40: 248-254, 2012

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Importance of Lymphoid System in juvenile studies

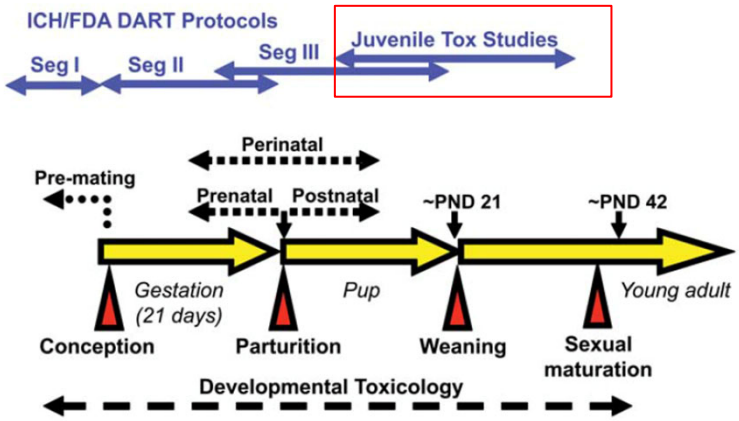

FIGURE 1.—Developmental toxicology timeline in the rat.

HOLSAPPLE AND O'LONE, Toxicologic Pathology, 40: 248-254, 2012

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Importance of Lymphoid System in juvenile studies

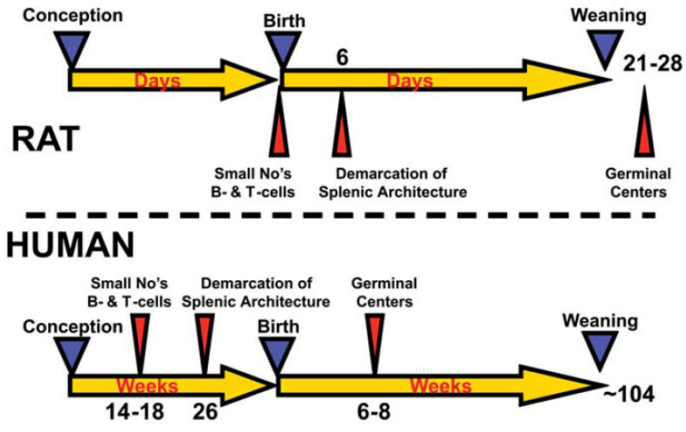

FIGURE 2.—Comparison of the development of the rat and human immune systems.

HOLSAPPLE AND O'LONE, Toxicologic Pathology, 40: 248-254, 2012

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Importance of Lymphoid System in juvenile studies

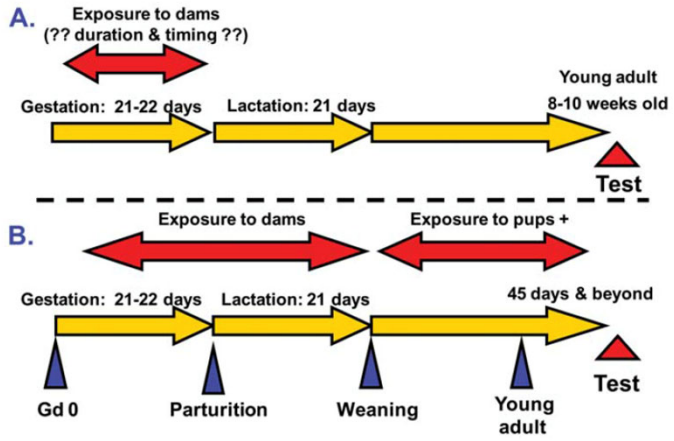

FIGURE 3.—Comparison of DIT protocols.

DIT = development immunotoxicity

HOLSAPPLE AND O'LONE, Toxicologic Pathology, 40: 248-254, 2012

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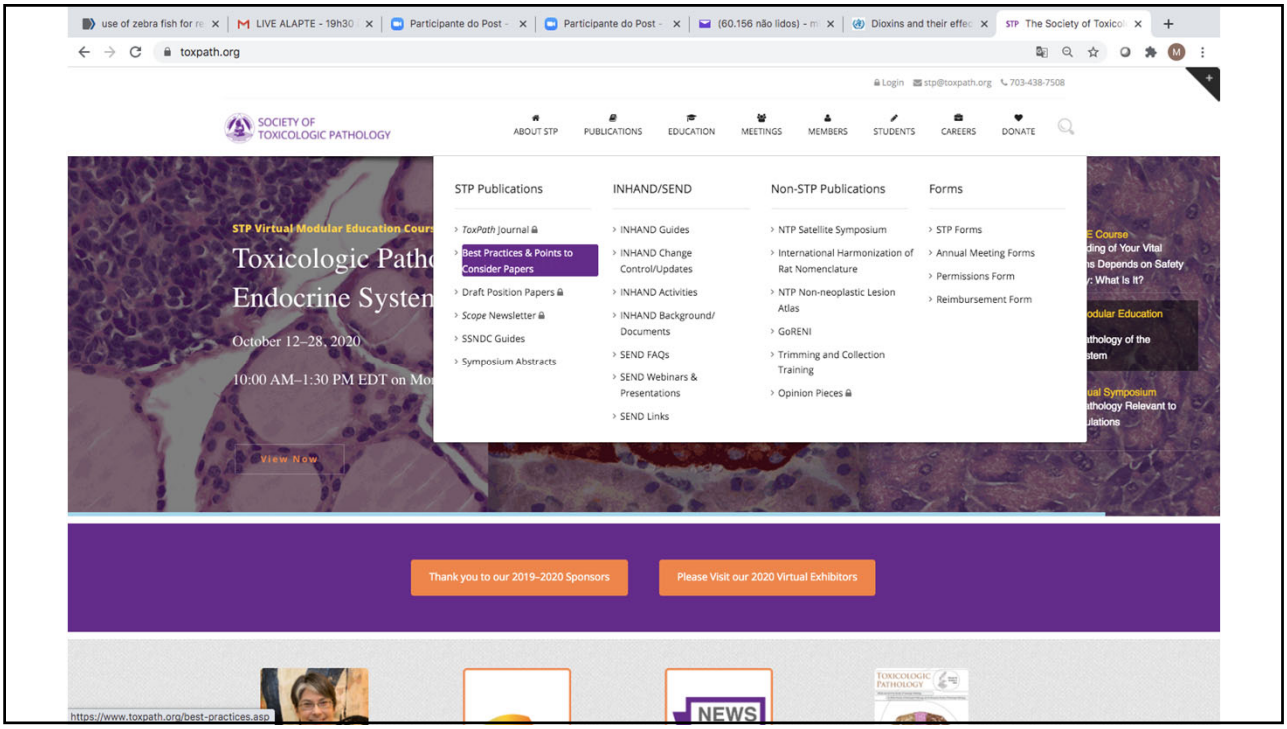

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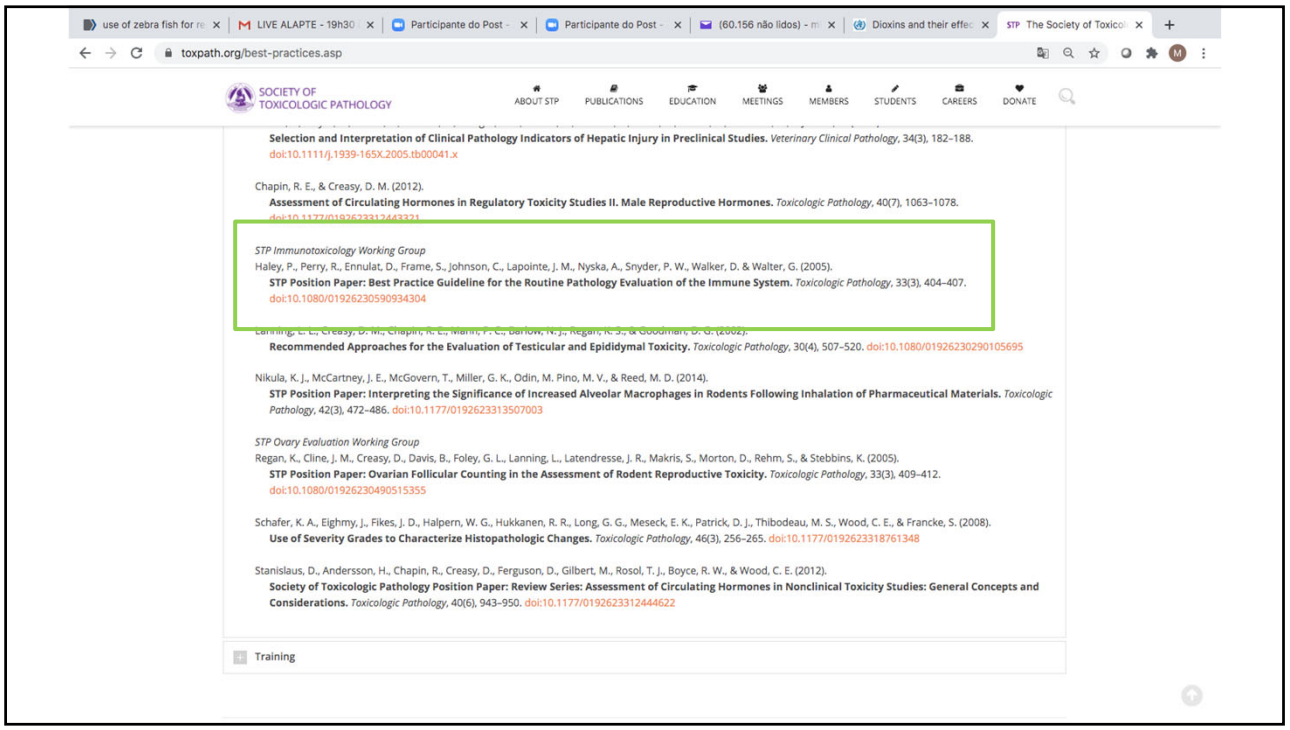
toxpath.org/best-practices.asp
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Selection and Interpretation of Clinical Pathology Indicators of Hepatic Injury in Preclinical Studies. Veterinary Clinical Pathology, 34(3), 182–188. doi:10.1111/j.1939-165X.2005.tb00041.x
Chapin, R. E., & Creasy, D. M. (2012).
Assessment of Circulating Hormones in Regulatory Toxicity Studies II. Male Reproductive Hormones. Toxicologic Pathology, 40(7), 1063–1078.
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Haley, P., Perry, R., Ennulat, D., Frame, S., Johnson, C., Lapointe, J. M., Nyska, A., Snyder, P. W., Walker, D. & Walter, G. (2005).
STP Position Paper: Best Practice Guideline for the Routine Pathology Evaluation of the Immune System. Toxicologic Pathology, 33(3), 404–407. doi:10.1080/01926230590934304
Recommended Approaches for the Evaluation of Testicular and Epididymal Toxicity. Toxicologic Pathology, 30(4), 507–520. doi:10.1080/01926230290105695
Nikula, K. J., McCartney, J. E., McGovern, T., Miller, G. K., Odin, M. Pino, M. V., & Reed, M. D. (2014).
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Regan, K., Cline, J. M., Creasy, D., Davis, B., Foley, G. L., Lanning, L., Latendresse, J. R., Makris, S., Morton, D., Rehm, S., & Stebbins, K. (2005).
STP Position Paper: Ovarian Follicular Counting in the Assessment of Rodent Reproductive Toxicity. Toxicologic Pathology, 33(3), 409–412. doi:10.1080/01926230490515355
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Training

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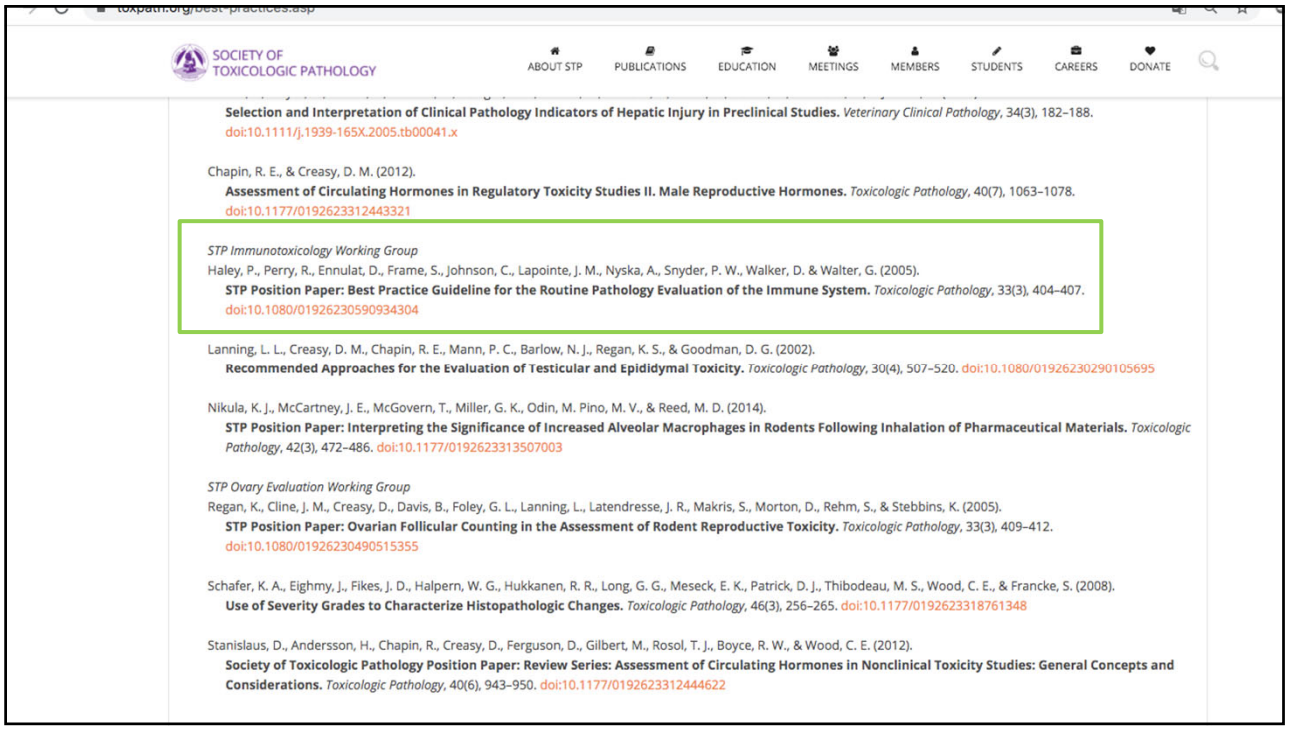
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Outline of the Presentation

1. Introduction
2. Ontogeny and Components of the lymphoid system
3. Evaluation of the lymphoid system
4. Immunotoxicity
5. Stress and the lymphoid system
6. The lymphoid system and juvenile studies.
7. Summary and conclusions

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7. Summary and Conclusions

- Lymphoid / immune system should be analyzed in standard toxicity studies (acute, subchronic, and DART).
- If required, lymphoid/immune system should be analyzed specifically with functional tests to evaluate immunologic function.
- Assessment of immunotoxicity is highly important in juvenile studies.

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