J Toxicol Pathol 2017; 30: 111–123

Review

The lymphoid system: a review of species differences

Patrick J. Haley[1*]

[1] Independent Consultant specializing in Immunotoxicology and Immunopathology, 852 Penns Way, West Chester, Pennsylvania, USA 19382

Abstract: While an understanding of the structure and function of a generically described immune system is essential in contemporary biomedicine, it is clear that a one-size-fits-all approach applied across multiple species is fraught with contradictions and inconsistencies. Nevertheless, the breakthroughs achieved in immunology following the application of observations in murine systems to that of man have been pivotal in the advancement of biology and human medicine. However, as additional species have been used to further address biologic and safety assessment questions relative to the structure and function of the immune system, it has become clear that there are differences across species, gender, age and strain that must be considered. The meaningfulness of these differences must be determined on a case-by-case basis. This review article attempts to collect, consolidate and discuss some of these species differences thereby aiding in the accurate placement of new observations in a proper immunobiological and immunopathological perspective. (DOI: 10.1293/tox.2016-0075; J Toxicol Pathol 2017; 30: 111–123)

Key words: species differences, lymphoid system, lymphoid function, immunology, immunobiology, immunopathology

Introduction

Immunotoxicology is a relatively young science developed to assist in understanding the impact of chemicals, especially environmental contaminants, on the immune system of man and animals[1–4]. However, regulatory guidelines designed originally to address low dose, chronic environmental and industrial contamination by chemicals were subsequently applied to the safety assessment of new drugs in the context of high-dose, short-term exposure, with conflicting outcomes. It has been during this time of expanded application of immunotoxicology concepts that a greater recognition and appreciation of the complexities of lymphoid tissue responses to injury and/or activation, as well as the significance of variable responses across species emerged.

The reader is encouraged to consult the many excellent articles and books available on general lymphoid anatomy and function of laboratory rodents and man. These include, but are not limited to: A Monograph on Histomorphologic Evaluation of Lymphoid Organs[5], *Histopathology of Preclinical Toxicity Studies*[6], *Atlas of Experimental Toxicological Pathology*[7], *Histology for Pathologists*[8], and others referenced herein[9–12]. Additional details concerning the collection and use of data derived from lymphoid tissues obtained from standard toxicology studies a can be found in the STP Best Practices: The Best Practice Guideline for the Routine Pathology Evaluation of the Immune System[13].

All lymphoid tissues, regardless of species, have a limited number of possible responses to tissue damage or stimuli. These include hyperplasia, hypertrophy, atrophy, necrosis, inflammation, and neoplasia. However, some histomorphological changes may reflect the normal function of that particular lymphoid tissue, such as filtering lymph, generating antibody, etc, and not pathology per se. Antigens translocated to lymph nodes typically result in immune stimulation reflected by cortical, paracortical, and/or follicular hyperplasia. Likewise, particulates may be translocated to, and accumulate in, lymph nodes leading to nonspecific reactive lymphoid hyperplasia. It is important to recognize that lymphoid tissues, especially lymph nodes and spleen, are not static organs and have resident and transient cell populations, as well as cells translocated to the lymphoid tissue from distant sites of tissue damage or antigen exposure. Recognizing which cell population within the individual lymphoid tissue compartment is affected is essential to understanding the significance of the observed histomorphological changes. Moreover, having a clear understanding for species differences in background lymphoid histomorphology that result from age, environmental exposure, gender, or response to pathogens is critical[14].

The histomorphology of normal lymphoid tissues, especially peripheral lymph nodes, can be highly variable and often overlaps with that of pathologically altered

Received: 30 November 2016, Accepted: 5 December 2016
Published online in J-STAGE: 24 December 2016
*Corresponding author: PJ Haley (e-mail: patrickjhaley@gmail.com)

tissues. Minor differences in collection, embedding, and sectioning combined with high intrinsic biological variability make consistent histological characterization and organ weight changes of lymph nodes problematic, and standardized collection and processing techniques mandatory[15, 16]. A historical data base of lymphoid organ weights should be developed and maintained for each species and strain of laboratory animal used, along with the age, weight and sex of the animals from which the data are collected. Each lymphoid organ has separate identifiable compartments that support specific immune functions and must be evaluated individually for changes. Semi-quantitative descriptive, rather than interpretative terminology, should be used to characterize changes within these compartments[13]. Lastly, it is essential that age, sex-matched controls are included in each study.

Thymus

The thymus is similar macroscopically and microscopically across species. Most larger mammals, including man, dog and nonhuman primates, have a bilobed thymus located in the anterior thoracic cavity just cranial to the heart and cardiac vessels. Differences seen in smaller mammals include a variable extension of one or both lobes into the cervical region in rats, a more anterior location in the neck region of guinea pigs[17] and a cervical as well as a thoracic location for the mouse[18]. The thymus of minipigs is also located in the thoracic cavity over the pericardium with extension into the thoracic inlet, and while also bilobed, the two lobes continue along the left and right jugular grooves in close association with the carotid artery up to the pharyngeal region, where it is referred to as the cervical thymus[19]. The thymus has a thin connective tissue capsule that surrounds the organ and penetrates into the lobes dividing it into multiple lobules. Lobules are most easily identified in larger species such as dog or monkey, but no lobular separation is seen in the mouse.

Histologically the thymus consists of an outer cortex of darkly-staining, densely-packed small lymphocytes that surround a clearly delineated inner, pale medulla. The medulla is usually continuous between adjacent lobules but and can appear to have distinct islands surrounded by cortex, depending on the histological sectioning[20]. Concentric whorls of flattened, eosinophilic, epithelial-derived reticular cells called Hassall's corpuscles, are visible within the medulla.

Thymic aging is very apparent in dogs, monkeys and humans and is characterized by involution and/or atrophy, as functional tissue is replaced by adipose tissue, interlobular stroma and epithelial structures (cords and tubules), especially of the medulla, become prominent. Involution is a biologically programmed physiologic elimination of thymic lymphocytes that is hormonally coordinated over the lifespan resulting in involution at the time of sexual maturity in most species[21]. Thymic lymphocytes are eliminated by apoptosis and phagocytic removal of cell debris from the cortex, resulting in a "starry-sky" appearance because of increased numbers of tingible body macrophages (macrophages containing 'tingible', or stainable apoptotic debris within their cytoplasm)[22].

The strain and species of the animal being tested also impacts the histomorphologic presentation of involution. For example, in general, female rats have prominent epithelial structures compared to males[23]. However, aging Wistar and WAG female rats have lymphocyte masses with limited epithelial components, and the thymus of aging Brown Norway female rats consists predominantly of cords and tubules with few lymphocytes. Highly variable thymic involution is seen in dogs undergoing sexual maturation and a wide range of thymic development and atrophy in normal dogs at 9–12 months of age is common. Nonhuman primates also have significant thymic variability influenced by the source of the animals (wild-caught versus purpose bred), age, and degree of sexual maturity. Increased numbers of adipocytes and more obvious interlobular stroma become apparent in both NHP and dogs as involution progresses.

Keratinization of Hassall's corpuscles is prominent in young dogs, nonhuman primates and minipigs but is less prominent in rats and rarely in mice. Premature keratinization may be seen as a component of xenobiotic-induced involution/atrophy in mouse, rat, dog and NHP (personal observations). Increased numbers and size of keratinized Hassall's corpuscles in rats can be striking and accompanies cortical changes of increased lymphocyte apoptosis and accumulation of cellular depletion.

Cystic embryonic remnants of the medulla are frequently seen in cynomolgus monkeys, but less so in rats and dogs. Epithelium-free areas (EFA) are subcapsular accumulations of darkly staining lymphocytes that appear darker than the adjacent cortex[23, 24] accompanied by many tingible body macrophages that present as 'holes' and can be easily mistaken for areas of increased cortical apoptosis. EFA are frequently seen in rats, and may be abundant and/or large in normal beagle dogs. Because they lack epithelial cells, EFA appear to be areas in which lymphocytes bypass stromal cell-mediated selection processes and move between the medulla and cortex without epithelial cell contact[25]. Other aging changes that are generally similar across species, including man, include cystic dilatation of Hassall's corpuscles with accumulation of cellular debris, dystrophic calcification, and the accumulation of foamy macrophages.

The human thymus begins the process of involution in the first 10 years of life and largely consists of fat and connective tissue by the time a human reaches the age of 30. In comparison, the rat thymus is robust at the ages at which most acute and subchronic repeat-dose toxicity studies are performed. It may proceed through complete involution during chronic studies[21] but can undergo complete recovery in a standard study recovery period even when the initial insult is severe (personal observation). While the human thymus is essentially absent in adults, alterations of the thymus in laboratory species remains a useful indicator of systemic effects of a test article on the immune system[13].

Detectable weight changes of the thymus frequently

precede histomorphologic changes[26, 27], but as with all lymphoid organs, weights can be problematically variable. The median thymic weight of a sexually mature normal male or female Sprague-Dawley rat may vary as much as 70% or more, and that for a normal male or female beagle dog, by approximately 120–170%[22].

Spleen

Early studies of the spleen resulted in the functional separation of the spleen as having either a defensive, storage, or intermediate role[28] with hematopoietic, lymphopoietic, immunologic and hemodynamic (blood storage, filtration) activities occurring variably across species. The vast amount of data derived from ultrastructural, histomorphological, immunohistochemical, *in vivo* and *in vitro* studies cannot be covered in this review. Instead, a generic description of the anatomic components (red pulp, white pulp, capsule and trabeculae) will be followed by descriptions of species differences in each that might indicate meaningful functional differences.

Capsule and Trabeculae

In general, the spleen can be viewed as a semi-elongated, distensible organ containing white blood cells, red blood cells and parenchyma surrounded by a fibro-muscular connective tissue outer capsule that penetrates as irregular trabeculae into the core of the organ. The density, thickness and relative abundance of the capsule and trabeculae contribute to identifiable species differences as noted below.

Red Pulp

A three-dimensional meshwork of splenic cords and venous sinuses are present in all species[29]. The splenic cords consist of reticular cells with associated fibers, and macrophages, that together filter blood and trap effete red blood cells (RBCs) and bloodborne particulates, iron pigment (hemosidirin), ceroid, and lipofuscin in the red pulp and marginal zone (MZ).

The complex vasculature of the spleen is central to the successful filtration of blood and recycling of RBCs. The blood enters the spleen at the hilus and flows sequentially as follows: splenic artery → trabecular arteries→small arteriole branches→red pulp→central arterioles→small arteriole branches→white pulp capillary beds with termination at the marginal sinus, in the marginal zone, or in the red pulp. Penincillar arteries and small arterioles move blood through the MZ and into either the venous sinuses (90%), or the reticular meshwork[30, 31].

White Pulp: PALS and Lymphoid Follicles

The white pulp lymphoid compartments include the periarteriolar lymphoid sheaths [PALS], primary and secondary follicles, marginal zone, and mantle, all of which varies across species. Identification and characterization of each splenic compartment, including evaluation of the relative size and cellularity of the periarteriolar lymphoid sheaths (PALS), the size and maturation of lymphoid follicles, the presence or absence of marginal zone cells, and the relative number of smaller lymphoid aggregates, are key for and accurate assessment of immunological impact on the spleen.

The PALS consist of dense accumulations of small, darkly stained (H&E) lymphocytes that surround and extend along the splenic central arteries. These accumulations can be further separated into a T-cell dependent inner zone consisting mostly of CD4+ T-cells accompanied by low numbers of CD8+ T-cells and interdigitating dendritic cells. An outer zone of the darker staining (H&E) outer PALS is made up of small CD+3 T-cells, B-cells, macrophages and occasional plasma cells[32]. Lymphoid follicles are present at the bifurcation of central arterioles and blend with the PALS[33].

The marginal zone (MZ) is a highly ordered and functionally distinct region that separates the red pulp from the white pulp, and consists mostly of B cells, MZ macrophages (MZMs; located at the outer side of the MZ) and marginal metallophilic macrophages (MMMs) found at the inner side of the MZ. The origin of MZ is complex. These cells arise from bone marrow cells committed to the B cell lineage, move to the spleen and become transitional B cells which then mature into either follicular B cells, or, while still in red pulp venules, into MZ B precursor (MZP) cells[34, 35]. MZ B cells do not recirculate as do B cells from lymph nodes, but will migrate into the white pulp, and transition to lymphoid follicles after exposure to bacterial products, such as lipopolysaccharide (LPS). MZ B cells contribute to natural immune responses and act primarily in initial antibody responses in support of T-cell independent humoral immune responses[36, 37]. Thus a loss of MZ lymphocytes may translate into a decline in T-cell independent antibody responses. Malaria and other infections can rapidly deplete MZ B cells in mice[38]. Marked decreases in MZ lymphocytes have also been noted in rodents, NHPs and dogs following dosing with xenobiotics, as well as in conditions of non-specific stress (personal observations).

Lastly, there are small irregular aggregates of small darkly stained lymphocytes scattered throughout the red pulp that should be evaluated for cellular density in comparison to controls.

The Defensive Spleen

Classically, extensive PALS, numerous lymphoid follicles, a thin capsule and thin trabeculae, both with limited contractile capability, characterize the defensive type of spleen. The predominantly lymphoid architecture of a defensive spleen is designed to mount immunologic defense, instead of being primarily a blood filtration or storage organ (Fig. 1). The defensive spleen is found in humans, NHPs, mice, rats and rabbits.

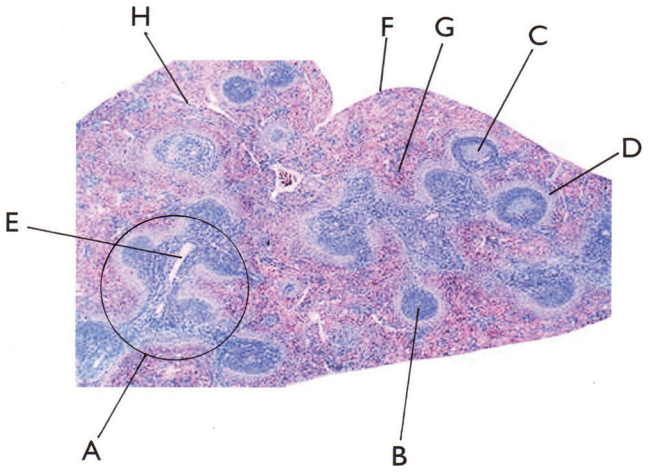

Fig. 1. Rat spleen. A: white pulp (PAS); B: primary folliculo-nodule; C: secondary folliculo-nodule; D: marginal zone; E: central arteriole; F: thin capsule; G red pulp; H: thin trabeculae.

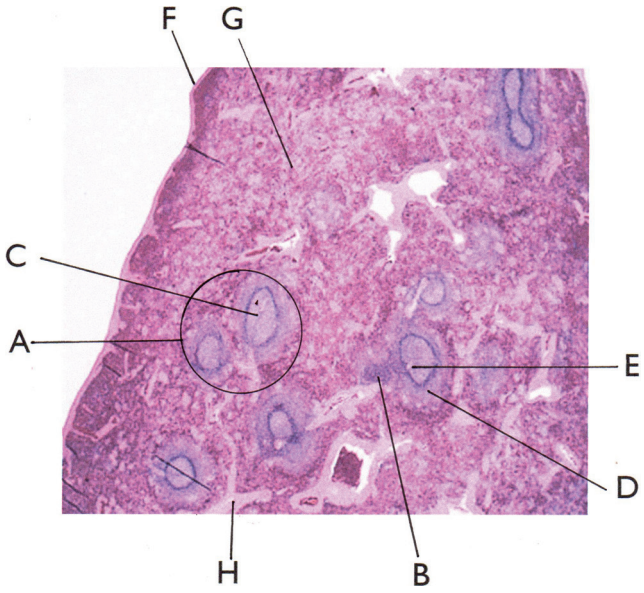

Fig. 2. Dog spleen. A: white pulp (PAS); B: primary folliculo-nodule; C: secondary folliculo-nodule; D: marginal zone; E: central arteriole; F: thick capsule; G red pulp; H: thick trabeculae.

The lymphoid follicles, marginal zone, and related structures are readily delineated in the rat but are less so in the mouse[33] and the PALS and the marginal zone seen in the rat and mouse are less distinct in humans[29, 39] and cynomolgus monkeys. The distinct marginal sinus that separates the marginal and mantle zones in the rat appears to be lacking in humans. In contrast to nomenclature for rodents, the term *marginal zone* in humans describes a specific and unique splenic structure that surrounds small IgD^+ and IgM^+ lymphocytes of the mantle zone or primary follicles[39]. In addition, the T cell areas of man are not as regularly arranged around arterioles as they are in rodents but instead occur as irregular areas containing small, polymorphic T-helper/inducer lymphocytes. The marginal zone in rodents contains specialized macrophages that include MMM (metallophils) and specialized MZ macrophages[40] but there is no mention of these specialized macrophage populations in humans[39].

The defensive spleen of NHPs present with numerous well-formed primary and secondary lymphoid follicles. Large, variably-shaped and coalescing lymphoid follicles with irregular and bizarre germinal centers are often seen in NHP. Such nodular hyperplasia of the white pulp, may be marked resulting in compression of the surrounding tissue, or diffuse. In some follicles the centers may contain an amorphous eosinophilic material, possibly a result of persistent antigenic stimulation and the deposition of putative antigen-antibody complexes. These variable and exuberant lymphoid follicles may be the result of chronic parasitic (including malaria), bacterial or viral infection and can occur in both wild-caught and purpose-bred cynomologus monkeys[41].

The Storage Spleen

In comparison to the defensive type spleen, the storage spleen, found primarily in dogs, is characterized by the presence of a thick outer capsule with many trabeculae of well-developed smooth muscle penetrating the parenchyma (Fig. 2). The smooth muscle allows the spleen to be contractile so that in addition to functioning as a blood-filtering organ, it can store up to 1/3 the circulating blood volume and can be rapidly emptied. The storage function vs a defensive function of this type of spleen is reinforced by the relatively small and localized PALS and fewer, sometimes poorly formed folliculo-nodules[15, 22, 42].

The Intermediate Spleen

Trabecular and lymphoid development intermediate to the other two types of spleen is found in ruminants and minipigs. Intermediate type spleens have a thick capsule of interwoven smooth muscle and elastic fibers with a moderate number of similarly heavy muscular trabecular penetrating deep into the parenchyma. The lymphoid components of the minipig spleen have small, less distinct lymphoid follicles than rodents but well developed PALS[19]. Sheathed capillaries derived from the central red pulp arterial branches are surrounded by concentric layers of periarterial macrophage sheaths (ellipsoids) and are quite large and numerous in the marginal zone of minipigs. Ellipsoids are also seen in dogs but not rats. Also, there are wisps of smooth muscle throughout the red pulp in minipigs[42].

Minipig spleens are considered nonsinusal because of poorly-developed or absent sinuses. Dogs and rats appear to have a sinusal spleen but true sinusoidal lining cells are lacking in the mouse[43]. The venous sinuses of the rat, referred to as pulp venules, are larger and more easily identified than those of the mouse[44]. Rat and mouse have numer-

ous capillaries within the PALS while few are seen in dog PALS.

Because whole blood passes through the red pulp, circulating WBC can be found within spleens and should be considered background cells and reflective of species differences in circulating WBCs[14]. Depletion of darkly staining tissue lymphocytes may result in increased visibility of background cells. Therefore, caution must be used when interpreting a possible increase of indigenous and/or transient cell populations that actually reflect a decrease in splenic lymphocytes with increased visibility of background cells.

Hematopoiesis

The spleen is a major source of hematopoietic activity throughout life in mice as indicated by large numbers of myeloid and erythroid precursors in the mature mouse spleen. Splenic hematopoiesis is much reduced in rats as compared to mice and is more likely to be seen in younger untreated animals. However, under conditions of increased demand, such as bone marrow toxicity, systemic inflammation, neoplasia, or anemia the adult rat spleen can significantly increase extra medullary hematopoiesis (EMH)[45]. EMH is not seen in dogs or NHPs in toxicology studies, even when the bone marrow is a target organ for toxicity[46]. Humans and rabbits have little hematopoietic activity in the embryonic spleen and essentially none in the adult, except under pathologic conditions[39].

Lymphopoiesis

Lymphopoiesis is recognized as the major function of the adult spleen in most species[47], but spleens of humans over the age of 20 rarely have active germinal centers[39]. In mammals, newly-formed lymphocytes migrate from the spleen to bone marrow and then to T- and B-cell areas of Peyer's patches, lymph nodes, and tonsils, as well as to the intestinal lamina propria and intestinal epithelium (intraepithelial lymphocytes)[48]. Splenic lymphocytes that migrate to bone marrow transform into plasma cells. The bone marrow and the spleen appear to receive the largest number of recirculating lymphocytes[49].

Histomorphological alterations of the spleen include decreased or increased cellularity of the PALS, marginal zone and folliculo nodules, (with or without increased apoptosis and tingible body macrophages); atrophy, capsular fibrosis, inflammation and necrosis. Other possible lesions include amyloidosis, phospholipidosis, lipidosis (adipocyte infiltration), and pigmentation. Focal white pulp nodular hyperplasia, also called lymphohistiocytic hyperplasia, can occur spontaneously in F344 rats or secondary to treatment with xenobiotics[50]. The reader is strongly encouraged to review the manuscript by Suttie[51] for excellent photographic examples of spleen lesions.

Accessory spleens, sometimes referred to as ectopic spleens, spleniculi, or splenic nodules are occasionally seen in cynomolgus monkeys and humans [22, 39]. These may be embedded in or attached to, the pancreas. Also in the pig, the gastrosplenic ligament may contain accessory spleens.

As with thymic weight, splenic weight, especially relative to brain, is an important component in the analysis for immunotoxicity, and decreased spleen weight has been found to be a reliable indicator of systemic immunotoxicity in rodents, especially when combined with histomorphology[13, 15]. However, spleen weights of dogs are unreliable because of blood engorgement following the use of pentobarbital for euthanasia and/or incomplete exsanguination.

Lymph Nodes

The histomorphology of normal lymph nodes ranges from small, simple, bean-shaped organs to nodes of highly variable architecture within individuals and across species. The age, husbandry (SPF vs non-SPF), species and strain of animals, as well as the likelihood of antigen stimulation of the node being examined, must be considered. This information must then be combined with knowledge of the number, location and species-based anatomy of the specific draining lymph nodes and their regional lymphatics to properly determine if the changes observed are biological or pathological.

For example, the mouse has relatively few lymph nodes (approximately 22) organized into simple chains[52]. As species increase in size, lymph nodes become more numerous (e.g. approximately 450 in humans), larger, and are organized into increasingly more complex chains. As another example, the lung of the rat is drained by two posterior mediastinal nodes[53] while the dog has three-to-five tracheobronchial nodes[54], and in man, there are 35 or more tracheobronchial nodes classified into five separate groups[55]. The nodes of larger species also have an increased number of anastomoses of afferent lymphatic vessels as compared to those in smaller species[56].

The mouse lymph node, described as a relatively simple, bean-shaped, uniform lymph node with a peripherally located, continuous cortex surrounding the medulla, has for years been the lymph node blueprint for all species [52, 57, 58].

The functional anatomical dynamics of lymph nodes have been presented in many texts and are beyond the scope of this review; only a brief summary will be presented herein.

The cortex of the lymph node contains lymphoid follicles designated as primary and secondary follicles. Primary follicles are distinct, rounded aggregates of small, densely staining resting lymphocytes without the formation of germinal centers, indicating the absence of antigen exposure. The presence of secondary follicles with germinal centers composed of immunoblasts (large, pale-staining, activated B lymphocytes), indicate that an antigen has been presented by antigen-presenting cells, such as dendritic cells (dendritic reticulum cells and interdigitating reticular cells), or T lymphocytes. Subsequent mitoses and differentiation into plasma cells or small memory B lymphocytes ensues. The memory B lymphocytes ultimately locate in the mantle zone

of secondary follicles and are long-lived. T lymphocytes migrate to, and locate in, the paracortical zone of the node where they participate in both cell-mediated and humoral-mediated immune responses; the remaining cortical cells are predominantly B lymphocytes. The medullary cords are composed of packed lymphocytes and numerous plasma cells. Around the cords are medullary sinuses that join efferent lymphatic vessels that conduct lymph away from the node. Apoptosis of lymphocytes that results in a starry-sky appearance in active nodes is most frequently seen in the dark zone of the follicle, but can be seen in the pale germinal center as demand increases for antibody production. The reader is encouraged to review other relevant articles[59, 60].

While generally similar to lymph nodes of the mouse, those from other species frequently have an extension of the medullary sinus into the subcapsular sinus resulting in highly variable segmentation. 'Functional complexes' within lymph nodes consisting of irregular, semi-rounded units with a central, dense lymphocyte population surrounded by a loose population of lymphocytes, reticulum network, postcapillary venules and lymphatic sinuses have been described[61-63]. These complexes may be single, or multiple and fused into large units with multiple follicles and a single expansive bulge of paracortical lymphocytes extending into the medullary space (Fig. 3). Larger species have an increase in the number of these irregular functional units rather than an increase in the size of individual units (Fig. 4) [64].

Lymph nodes from specific pathogen free (SPF) rats and mice tend to have small numbers of primary follicles and few secondary follicles because of low-level antigen exposure. But even in SPF animals, lymph nodes that drain tissues with localized exposure to microbes and pathogens, such as the mandibular and mesenteric nodes, frequently contain large secondary follicles with germinal centers, wide medullary cords filled with plasma cells (e.g.mandibular nodes) and/or sinuses filled with macrophages (e.g.sinus histiocytosis of mesenteric lymph nodes). Alternatively, non-SPF dogs, swine, or NHP are likely to have larger and more numerous secondary follicles because of increased environmental pathogen or antigen exposure [14, 19, 41].

The histomorphology of both normal and pathologically altered lymph nodes can be remarkably confusing. It is essential that all of the work by Belisle and Sainte-Marie be thoroughly reviewed in order to correctly identify, characterize and understand lymph node alterations[61-64]. As stated previously, histomorpological variability can be exaggerated by collection and sectioning techniques[15, 16]. Because lymph nodes do not have a simple, consistent shape, serial sections of a node can reveal areas of markedly different proportions of cortex, paracortex and medulla[61, 62].

All species, including humans, develop significant age-related changes as the node atrophies and becomes replaced by fibrous connective tissue and fat. Residual hematopoietic activity can be found in rodent but not human, dog or NHP lymph nodes.

Minipigs have an anatomical reversal of the classical lymph node cortical and medullary compartments as well as the location of afferent and efferent lymphatics (Fig. 5)[47]. Lymph in minipigs flows into the node centrally and leaves the node through efferent vessels located on the capsular surface. Likewise, the medullary sinusoids and cords are located peripherally, while paracortical T cell-dependent areas and the lymphoid follicles are located centrally within the node. Large irregular medullary sinusoids may extend into the center of the node or fill the majority of one pole of the node[19].

As has already been stated, many of the changes identified in lymph nodes in toxicology studies reflect normal function and not pathology per se, such as cortical hyperplasia, sinus histiocytosis, sinus erythrocytosis, and accumulation of particulates[65].

Reactive lymphoid hyperplasia (RLH), is a common histomorphological response of lymph nodes that can be specific (e.g. antigen driven by viruses or bacteria) or non-specific (e.g. driven by chemical pollutants, tissue damage) involving one, several, or all of its anatomic subunits. RLH may have a follicular, sinus, diffuse or mixed pattern that if severe, may efface the nodal architecture[58]. Resident cell populations as well as migrating cell populations may be involved in RLH as a response to local or systemic conditions. Mucosal and cutaneous sites are particularly prone to localized inflammation that impacts local draining peripheral lymph nodes. For example, mandibular and popliteal nodes in both SPF and non-SPF animals, often show marked RLH as a background response to antigens and/or nonspecific irritants encountered through mucocutaneous or skin surfaces. Cage sores and pododermatitis are often seen in caged beagle dogs, accompanied by inflammatory infiltrates and RLH of the popliteal and/or axillary nodes[66]. Comparatively, rodents tend to have RLH of mandibular nodes with plasma cell accumulation in medullary cords secondary to dental disease (malocclusion, broken incisors)[67, 68].

Bacterial, fungal or parasitic infection/infestation (i.e. demodectic mange) of lymph nodes of dogs can result in partial or total effacement of nodal architecture by granulomatous and pyogranulomatous inflammation (personal observation). Mange mites and hair shafts can be found within lymph nodes and afferent lymphatics draining severe skin lesions associated with demodectic mange (personal observation). Granulomatous inflammation of the node should not be confused with sinus histiocytosis, which is the accumulation of vacuolated/foamy-appearing macrophages within sinusoids, and is considered a normal finding for mesenteric lymph nodes of most species[69]. Nevertheless, an increase in incidence and severity of any background lesion may indicate a treatment effect by a xenobiotic.

Amyloidosis of lymph nodes, characterized by the accumulation of a homogenous pale eosinophilic, Congo red positive, material in the subcapsular sinus and paracortex, occurs in mice (especially CD-1), NHP, but not in rats. Amyloidosis is a systemic condition and other lymphoid tissues, such as the spleen may also contain similar deposits. Amyloidosis is rare in purpose bred beagle dogs but may be associated with juvenile polyarteritis that may include in-

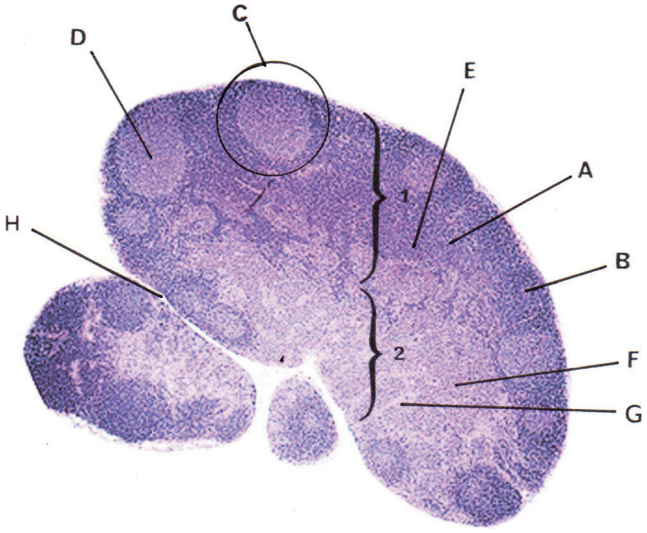

Fig. 3. Rat lymph node. 1: Cortex; A: peripheral cortex; B: primary folliculo-nodule; C: secondary folliculo-nodule; D: germinal center; E: deep cortical unit; 2: Medulla; F: medullary cord; G: medullary sinus; H: septum.

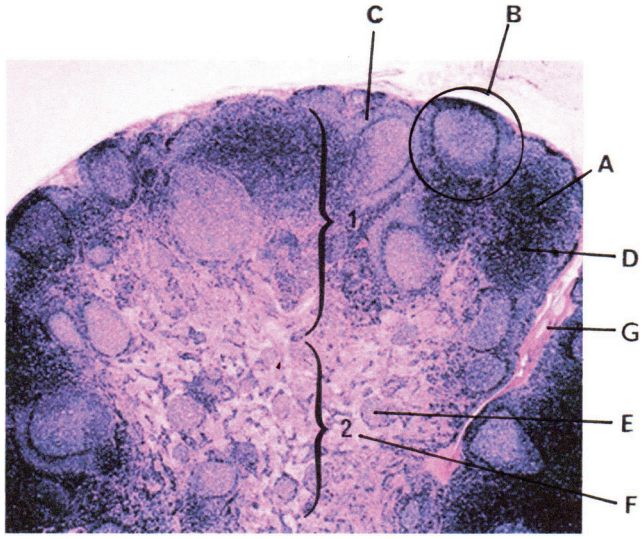

Fig. 4. Dog lymph node. 1: Cortex; A: primary folliculo-nodule; B: secondary folliculo-nodule with germinal center; C: mantle; D: deep cortical unit; 2: Medulla; E: medullary cord; F: medullary sinus; G: septum.

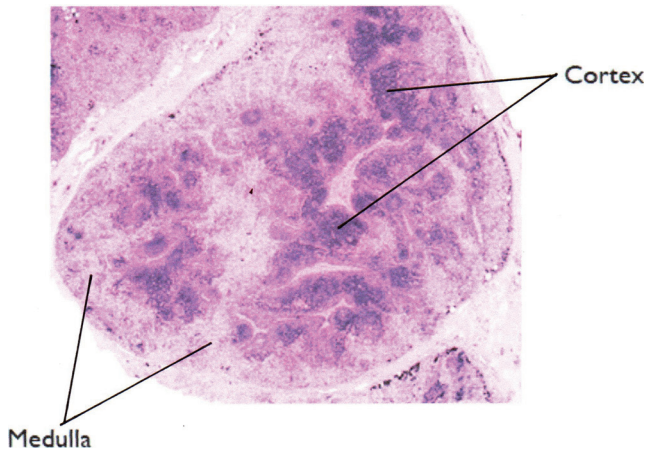

Fig. 5. Pig lymph node. Note reversal of location of cortex and medulla.

volvement of the spleen[70]. It's presence in mice is genetically driven, but in NHP chronic antigen-antibody responses and/or chronic inflammation are suspected.

Large numbers of eosinophils frequently occur in the peripheral (mandibular, axillary, popliteal), but not central (mesenteric, hepatic) lymph nodes of dogs likely as a result of clinically silent demodectic mange mite infestation that is common in most dogs, including purpose-bred beagle dogs. This author has identified occasional demodectic mange mite granulomas in the peripheral nodes of control dogs with no clinical evidence of demodecicosis. Thus the eosinophil infiltrates within peripheral nodes reflect the functional aspect of a regional draining node and is not an indication of nodal pathology (i.e. eosinophilic lymphadenitis).

Mucosal Associated Lymphoid Tissue (MALT)

An essential component of the lymphoid system is the mucosal associated lymphoid tissue (MALT). MALT is comprised of dispersed yet integrated aggregates of non-encapsulated lymphoid tissue within the mucosa that maintain immune responses at mucosal surfaces. MALT has numerous subcomponents that include the bronchus associated lymphoid tissue (BALT), nasal associated lymphoid tissue (NALT), and gut associated lymphoid tissue (GALT). Additional but less well reported representatives of MALT include conjunctiva-associated lymphoid tissue (CALT), larynx-associated lymphoid tissue (LALT) and salivary duct-associated lymphoid tissue (DALT), with more likely to be added[71-73]. BALT, GALT and NALT share anatomical and functional attributes that include distinct lymphoid follicles, interfollicular areas, a subepithelial dome, and an overlying follicle-associated epithelium (FAE or lymphoepithelium), with or without M cells (microfold cells). M cells are responsible for sampling luminal antigen from the mucosal surface and transferring it to antigen-presenting cells within the lymphoid tissue. Other than M cells and FAE, MALT is home to the typical cells of other lymphoid tissues, including B-cells, CD4+ and CD8+ T-cells, and dendritic cells. MALT does not have afferent lymphatics but HEV are present.

Bronchus Associated Lymphoid Tissue (BALT)

BALT, first described by Bienenstock *et al.*[71, 72], is organized bronchial lymphoid aggregates or follicles located within the bronchial submucosa of rabbits, rats, guinea pigs, mice, dogs, pigs, chickens and humans, often at the bifurca-

tions of airways. Definitive T and B cell areas have only been described in rabbit BALT[74]. Not all species have significant amounts of BALT[75]. Rabbits and rats have the most BALT, while mice and guinea pigs have an intermediate amount. BALT in man is identified as diffuse but limited collections of subepithelial lymphoid tissue in bronchi with only some of BALT in small bronchi and bronchioles. Human BALT is organized similarly to that in rabbits. Application of SPF conditions has resulted in marked decreases in BALT of normal healthy rodents, especially rats and reliable identification of compound effects on BALT requires rigorous sampling techniques.

Species differences concerning pulmonary immune responses have been described and the details are extensive[76–83]. The reader is encouraged to review these and other references for a greater understanding of differences of pulmonary immune responses across species.

Nasal Associated Lymphoid Tissue (NALT)

NALT is defined as focal submucosal aggregates of lymphoid cells within the nasal cavity. Similar to other forms of MALT, NALT has FAE and M cells. Specific surface markers are expressed by M cells that act as antigen sampling sites within the nose[84, 85]. While NALT in rats appears to be restricted to the ventral aspects of the lateral walls at the opening of the nasopharyngeal duct, NALT in NHP is more prominent and is located on both lateral and septal walls of the proximal nasopharynx[86]. The pharyngeal tonsil (adenoid) of man is part of NALT along with the less prominent collections of submucosal lymphoid follicles on the nasopharyngeal surface of the soft palate, in the lateral and posterior wall of the nasopharynx, and around the orifice of the eustachian tube[87].

The NALT of mice, rats, and hamsters is functionally and structurally similar[88–92]. This author and others have identified NALT in normal dogs that is anatomically similar to other species. NALT can be a target organ for immunomodulatory drugs as indicated by a reduction of NALT cellularity following the nose-only inhalation of corticosteroids by dogs[93].

Gut Associated Lymphoid Tissue (GALT)

GALT is one of the more commonly recognized forms of MALT in animals and man. It is characterized by discrete follicles of lymphocytes, [Peyer's patches (PP)] located within the mucosa along the free wall of the small intestine, but also includes large numbers of lymphocytes scattered throughout the lamina propria (LPL), intraepithelial lymphocytes (IEL), cryptopatches in the small intestine, and lymphoglandular complexes in the colon[94]. A specialized overlying epithelium (FAE) and M cells[95] are found in PP and actively sample antigen from the intestinal luminal contents and transfer antigenic material to lymphoid cells within the follicle[96]. Approximately 50% of the overlying cells in the rabbit are M cells whereas only 5–10% of the overlying cells are M cells in rat and human. Follicular B-cells and adjacent T-cell areas lie beneath the FAE.

LPL are numerous and may be equivalent to the spleen in overall mass. They synthesize IgA and can recirculate back to the point of origin. All components of GALT play a central role in primary mucosal tissue immune defenses and should be carefully inspected in all safety assessment studies.

While GALT varies in location and size depending upon the species, corresponding differences in function have not been determined. For example, the follicles of mice and rats are of uniform size throughout the small intestine with 6–12 follicles present in an aggregate[97], whereas that of pigs, dogs and ruminants have two distinct types of PP. These two types present as discrete patches in the jejunum and upper ileum, and as long continuous patches in the terminal ileum. Microbial exposure is not required for growth of jejunal or ileal PP in the pig and the numbers and size of follicles increase with age accounting for their increase in the length in the jejunum and ileum. Unlike the pig, the presence of bacteria is needed for development of PP in rodents[98]. The number of follicles in pigs may reach 75,000 by approximately 1 month of age, with the majority located in the ileum but the ileal PP regress to a few small scattered follicles in older animals[99]. The proximal PP of dogs, man and rodents are similar in appearance[100]. In man, PP follicles increase in size and number with age until puberty, but decrease thereafter. Duodenal patches in humans are small and contain few follicles that increase in size and number distally in the gut, achieving the largest size at the ileocecal valve. The terminal ileal PP in humans may contain 900–1000 individual follicles at maturity[98].

The PP is the source of IgA positive lymphocytes in rabbits and rodents. These cells leave via the blood and lymph to mature in mucosal and glandular sites[100]. The domes of PP in pigs are devoid of IgA plasma cells, and few IgA positive cells are found in the PP of man or rat[101]. The domes in the human appendix and the dome region of all dog PP have many IgG plasma cells, but far fewer are present in the domes of rodent[100]. Rats have T-cells, IgA, IgG, IgM positive B-cells, and Ia positive cells at birth. The sacculus rotundus is a distinct hypertrophied Peyer's patch that encircles the terminal ileum in man. Both humans and rabbits have an appendix, a large aggregation of lymphoid nodules at the ileocecal valve. An appendix is absent in mice and rats, but they have large aggregations of lymphocytes in the walls of the cecum.

There are many single and aggregate lymphoid nodules in the colon and rectum that are especially prominent in the dog. Histologic changes in rectal lymphoid aggregates of dogs must be interpreted with caution as self–induced trauma and/or pathology of the adjacent anal glands can result in considerable localized damage and inflammation (personal observation).

Orally administered immunomodulatory compounds can have dramatic effects on GALT as indicated by decreased lymphocytes and increased apoptotic cells within

the interfollicular zone and/or within lymphoid follicles. Decreases in GALT lymphocytes may be associated with the mucosal invasion by opportunistic microbes and/or parasites, especially in dogs and monkeys that may result in local mucosal erosion, ulceration, and systemic dissemination of pathogens. Even small foci of acute inflammation of PP may allow the entrance of microbes to regional mesenteric lymph nodes (personal observation). Recurring diarrhea from a number of bacterial species that include Camplyobacter spp., Shigella flexneri, Yersinia enterocolitica, adenovirus, and Strongyloides fulleborni is the leading cause of NHP morbidity[102]. Background levels of such infections can be worsened secondary to the stress of being put on a study, regardless of the chemical class of the compound. The regional draining lymph nodes, such as the mesenteric nodes, may also show histological evidence of microbe invasion with subsequent inflammation. A correlation between intestinal inflammation and involvement of mesenteric nodes should be determined. Because the gastrointestinal submucosa of dogs and NHPs usually have a high background of mixed inflammatory cell infiltrates, it can be very difficult to differentiate between normal levels of background inflammation and early low-grade effects of a test article, making careful scrutiny of tissues from controls and dosed animals crucial. Distinct GALT of the stomach (GGALT) may be particularly prominent in dogs and NHP if *Heliobacter sp.* is present[41]. Compound-related lesions of GALT can be especially challenging. Researchers are strongly encouraged to maintain a database of background lesions of the intestinal tract and the related GALT from dogs and NHP so as to more accurately place new observations in the proper context.

Bone Marrow

A detailed review of the considerable literature of the anatomy and biology of the bone marrow is beyond the scope of this article, and only a brief description will be provided here. The reader is strongly encouraged to review any of the exceptional references on the subject[103–107].

The marrow cavity has trabeculae of cancellous bone that are lined by a single layer of cells resting on a delicate layer of reticulum (endosteum) along with aggregates of osteoclasts and osteoblasts. Microvasculature is present but lymphatic channels are absent. The marrow also has a limited network of fine, branching reticulum fibers located between the parenchyma of erythroid, myeloid and lymphoid precursors and maturing cell types.

The bone marrow is the site of origin of pluripotent stem cells which give rise to lymphoid and erythroid stem cells. Bone marrow lesions include decreases or increases in all cells or specific cell subtypes, adipocyte infiltration, necrosis, infarction, fibrosis and hemorrhage. Age-related changes in rat bone marrow may be encountered that include hyperplasia, atrophy, and stromal proliferative changes such as myelofibrosis, myelosclerosis, fibrosis, osteosclerosis, osteopetrosis, and osseous metaplasia[105]. Decreases in lymphoid populations of the bone marrow typically parallel changes in other lymphoid tissues such as the spleen and thymus. Decreases in bone marrow cellularity are accompanied by an increase in fatty tissue resulting in the formation of 'holes' within the parenchyma. These 'holes'represent adipocyte infiltration, and are accompanied by small, scattered aggregates of apoptotic bodies. Decreased marrow cellularity, can be seen in conditions of chronic inflammation, neoplasia, hypothyroidism, hypoadrenocorticism, chronic renal or hepatic disease, and direct toxicity[104]. Outright bone marrow necrosis is seen with severe caloric restriction of 25% of control for at least 2 weeks[108] and bone marrow depletion has been reported with cachexia and severe debilitation[109]. For accurate assessment of which cell populations are specifically affected (erythroid, myeloid, or lymphoid) and to determine the myeloid to erythroid ratio as well as for the presence of any cytological abnormalities, it is recommended that an evaluation of bone marrow smears be performed.

Whereas the structure of the bone marrow is functionally the same across species, there are histological differences depending on the age, species, strain and the particular bone that is being examined (i.e. femur versus sternum). Rodents at the age typically used in acute and subchronic studies have abundant hematopoietic tissue in the femoral head, femoral shaft, sternum and rib. The amount of hematopoietic tissue declines noticeably in normal rodents over a 3–6 month period, thereby making it mandatory to only use age-matched controls for evaluating xenobiotic-induced alterations. However, young and adult dogs and nonhuman primates tend to have little-to-no hematopoietic tissue in the head of the femur and distal femoral shaft making these sections in larger animals of little use when determining bone marrow affects. Dogs and NHP are known to occasionally have variably-organized lymphoid aggregates, with and without follicular development, within the bone marrow. Such aggregates in NHP are postulated to be related to type D retrovirus infection[41]. Similar lymphoid cell aggregates with well-developed germinal centers in bone marrow of rats given test article have been observed.

Disclosure of Potential Conflicts of Interest: None

Note: All photomicrographs have been modified or altered to enhance descriptive qualities.

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