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Enhanced Histopathology of the Thymus

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Abstract

The thymus is a primary or central lymphoid organ in which T lymphocytes undergo differentiation and maturation autonomously within the cortex, without the need for antigenic stimulation, and it is essential for the normal development and function of the immune system. The thymus has been shown to be a sensitive target organ following exposure to immunotoxicants and endogenous corticosteroids, and a decrease in size or weight is often one of the first noted measures of compound-induced effects with cortical lymphocytes (thymocytes) being especially susceptible. Therefore, changes in thymus histopathology and architecture are considered to be of particular relevance for immunotoxicity screening. The separate compartments in each lymphoid organ should be evaluated separately and descriptive rather than interpretive terminology should be used to characterize changes within those compartments (Haley et al., 2005). Therefore, enhanced histopathological evaluation of the thymus involves the determination of the size and cellularity of the cortex and medulla, which should be noted separately. Other changes to evaluate include, but are not limited to, increased lymphocyte apoptosis, lymphocyte necrosis, cortex:medulla ratio and an increase or decrease in the epithelial component of the thymus.

Keywords

Apoptosis; cortex; medulla; tingible body macrophage; stress response

Introduction

The thymus has been shown to be a sensitive target organ following exposure to immunotoxicants and a decrease in size or weight is often one of the first noted measures of toxicity (Schuurman et al., 1992). Moreover, lymphocytes (thymocytes) within the thymic cortex appear to be especially susceptible to the action of toxic compounds, both directly and indirectly (via the release of endogenous corticosteroids). Therefore, changes in thymus histopathology and architecture are considered to be of particular relevance for the determination of immunotoxicity (Van Loveren et al., 1996; Vos et al., 1997; Harleman, 2000; Kuper et al., 2000). Recent studies that examined the sensitivity of enhanced histopathology in the immune system of B6C3F1 mice demonstrated that the most consistent and discernable lesions were noted in the cortex of the thymus (Germolec et al., 2004).

It has also been shown that the histological findings in the thymus associated with a variety of different pharmaceutical agents correlate well with thymus weight and peripheral lymphocyte counts in both the rat and dog (Wachsmuth, 1983). However, since the thymus is an organ that is sensitive to the effects of stress (endogenous corticosteroids) and aging, it is very important to differentiate chemical-induced thymic atrophy from stress-related lymphocyte apoptosis and

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age-related thymic involution. Due to the potential difficulties of differentiating age-related changes from chemical-related effects, it may be best to conduct enhanced histopathology of the immune system in shorter-term studies, such as 14-day, 28-day or 3-month bioassays. As with all histopathological evaluations, comparison with control tissues is crucial.

According to the STP position paper: Best Practice Guideline for the Routine Pathology Evaluation of the Immune System (Haley et al., 2005), the separate compartments in each lymphoid organ should be evaluated separately and descriptive rather than interpretive terminology should be used to characterize changes within those compartments. Therefore, enhanced histopathological evaluation of the thymus involves the determination of the size and cellularity of the cortex and medulla, which should be noted separately. Refer to Pearse for more detailed information on the normal structure and function of the thymus (Pearse, 2006).

Decreased Cellularity

Decreased cellularity of the thymus is the most frequently encountered histologic finding associated with compound-induced effects on the thymus. Because decreased cellularity is often associated with the histologic presence of dead lymphocytes it is important to attempt to distinguish between lymphocyte apoptosis versus necrosis. The presence, severity grade and location of cell death, when present, should be determined. The determination of the type of cell death is important because it may provide insight into the pathogenesis of the lesion. The STP Committee on the Nomenclature of Cell Death recommends the use of the term “necrosis” to describe findings comprising dead cells in histological sections, regardless of the pathway by which the cells died (Levin et al., 1999). They also recommend the use of the modifiers “apoptotic” and “oncotic” to specify the predominant morphological cell death pathway.

Oncotic necrosis is the cellular process that can be seen in areas of thymus infarction or as a direct treatment-related effect and may or may not be accompanied by an inflammatory response rich in neutrophils. With oncotic necrosis, there is cell swelling and rupture of the cell membrane and subsequent release of cytoplasmic contents into the surrounding interstitium which incites the inflammatory response. Apoptotic necrosis on the other hand, is characterized by cell shrinkage, nuclear fragmentation, extrusion of membrane-bound cytoplasm and nuclear debris in the form of small dense apoptotic bodies. This process is typically accompanied by tingible body macrophages (defined as macrophages containing stainable cellular debris), which give the tissue a “starry sky” appearance (Figure 1c). It is therefore recommended that, whenever possible, a diagnosis of lymphocyte oncotic necrosis be reserved for those cases where treatment results in the classical form of necrosis rather than apoptosis.

As noted before, decreased numbers of lymphocytes in the thymus, leading to decreased cell density, decreased cellularity, or decreased compartment size, may be the result of direct thymic lymphocyte toxicity or may result from endogenous glucocorticoid release or age-associated involution. Certain chemicals that lead to direct thymus lymphocyte toxicity may result in increased numbers of apoptotic lymphocytes and tingible body macrophages (Figures 1 and 2) or may result in oncotic necrosis (Figure 3). While not an absolute truth, apoptotic necrosis is most likely to occur as a secondary response to stress, while that of oncotic necrosis may be considered to be more representative of direct lymphocyte (thymocyte) toxicity. However, apoptotic necrosis and oncotic necrosis are not mutually exclusive processes and thus may occur simultaneously since both represent morphologic expressions of a shared biochemical network (Zeiss, 2003).

Endogenous glucocorticoid release in response to stress and debilitation can occur within a group of animals and this can result in increased numbers of thymus cortical apoptotic lymphocytes. However, lymphocytes in the cortex normally undergo numerous cell divisions before entering the medulla and apoptosis is a normal but usually minimal finding in this

population of rapidly dividing cells. Therefore, an increase in the number of apoptotic cells should be noted only after comparison with controls. Although various methodologies are available for evaluating apoptosis (stained resin sections, DNA laddering, TUNEL, annexin V, caspase-3 activity as-says, mitochondrial assays, vital dyes and lysotracker red), each assay has its advantages and disadvantages that can render it appropriate and useful for one application but inappropriate or difficult to use in another (Watanabe et al., 2002). Transmission electron microscopy (Figure 2) is considered the “gold standard” for the evaluation of apoptosis.

Spontaneous aging and end-stage or chronic experimentally induced non-neoplastic thymic lesions are often morphologically similar with reduction in thymic weight and histological depletion of cortical lymphocytes. Therefore comparison with age-matched controls is crucial. The mechanisms responsible for age-related thymic involution are not known however sex hormones are involved. Orchidectomy in rats will cause involutonal effects on the accessory sex organs with trophic effects on the thymus due to removal of the sex hormones. Treatment of old male rats with a stable analogue of luteinizing hormone-releasing hormone (LHRH) and the subsequent decrease in testosterone has been shown to result in regeneration of the thymus (Greenstein et al., 1987). Estrogen also has significant immunomodulatory properties, including induction of thymic involution (Yao and Hou, 2004).

Cortex:Medulla Ratio

An increase or decrease in the cortex:medulla ratio is another parameter that can be determined and recorded. However, within each lobule, the plane of section results in variation of this ratio when measured at multiple points. Therefore, in order to obtain an accurate ratio, a qualitative assessment in which the average of multiple ratios is determined or a quantitative morphometric analysis of the cortical and medullary areas would have to be determined as described by Pulido et al. (2005). To qualitatively evaluate this ratio, the functional lobule with an outer cortex and inner medulla should be examined. In general, the medulla normally occupies about one-third of the lobular volume in a typical adult rodent. A normal cortex:medulla ratio would therefore be close to 2:1. However, due to tangential cuts that can occur within any given lobule, all lobules should be examined and an overall ratio determined (Figure 4). In general, moderate to severe changes can be easily detected and graded qualitatively but the minimal-to-mild changes can be difficult to assess but can be detected by quantitative morphometric measurements. As always, a careful comparison with controls is needed to accurately identify a no effect level for the change.

Increased Lymphocytes

An increase in the numbers of lymphocytes can occur within the cortex or medulla and can be focal or diffuse (Figures 5-7). These lesions should be distinguished from lymphoma and the focal lymphocyte hyperplasia that accompanies age-related thymic atrophy. An increase in lymphocyte numbers in the thymus is usually in response to antigenic stimulation, accompanying inflammation, tumors, etc., and the cell population is mixed in contrast to the more homogeneous neoplastic population.

Increased Epithelial Component

An increase in the numbers of epithelial cords and tubules within the medulla is another feature to evaluate (Figure 8). The loss of medullary lymphocytes can result in the epithelial component of the medulla appearing more prominent, but not necessarily with an increase in the number or size of epithelial cells. Since prominent and hyperplastic medullary epithelial cells are also a common age-related change found in association with thymus involution, consideration of age and comparison with controls should help to determine if this histological change is test

article or age-related. The proliferation of thymic epithelial cells should also be differentiated from the neoplastic epithelial component of thymomas in which the cells can be spindle-shaped, ovoid or polygonal in shape with large nuclei or undergoing squamous differentiation.

Epithelium-Free Areas

The epithelium-free areas (EFA) in the thymus are lymphocyte-rich regions, devoid of stromal elements, non-vascularized, and with unknown function (Figure 9; Bruijntjes et al., 1993). It is postulated that they may be lymphocyte reservoirs (Van Ewijk, 1984), proliferation sites of lymphocytes (Duijvestijn et al., 1982; Godfrey et al., 1990) or a specific intrathymic pathway for T lymphocytes (Bruijntjes et al., 1993). These EFAs are located in the subcapsular region and serial sections show that they run from the subcapsular area to deep in the cortex, often bordering the medulla (Bruijntjes et al., 1993). The occurrence and extent of EFAs varies between rat strains. They have been identified in the Wistar rat, diabetes-prone and diabetes-resistant rats however they have not been identified in the WAG/Rij rats. The occurrence and extent of EFAs can also vary with age. In Wistar rats EFAs are extensive whereas in rats over 17 months of age these areas have not been found. Some EFAs may be evaluated during the initial H&E screen however many EFAs may be overlooked using this method alone (F. Kuper, personal communication). Therefore, for an in-depth examination to allow for the accurate identification and evaluation of this compartment, special stains would be needed. Keratin and laminin stains for stromal elements would be negative and a stain for MHC class II cells would be negative except for a few cells (Bruijntjes et al., 1993).

Additional Cells and Lesions

The presence, severity grade and location of other cells and lesions, such as pigment, extramedullary hematopoiesis, cysts, etc. should also be noted. An example of a checklist for the changes to be noted in the thymus for enhanced histopathology is given in Table 1. This table is intended to be an example of a guideline, but not a rigid checklist, that the pathologist can use during histological evaluation rather than a format for reporting lesions. The diagnoses listed in this table are descriptive rather than interpretive, consistent with the STP position paper: Best Practice Guideline for the Routine Pathology Evaluation of the Immune System (Haley et al., 2005).

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Figure 1.

Figures 1a and 1b are low and high magnifications of normal thymus cortex from a control 30-day-old male Sprague–Dawley rat. These can be compared with the low and high magnifications of thymus cortex in Figures 1c and 1d from an age- and sex-matched rat treated 3 hours previously with 1 mg/kg bodyweight of dexamethasone. In the control thymus there are only rare apoptotic bodies (arrow, Figure 1b). In the images from the treated rat, there are increased numbers of apoptotic lymphocytes and tingible body macrophages (macrophages containing stainable bodies or cellular debris) giving this tissue a classic “starry sky” appearance at low magnification (Figure 1c). The arrows in Figure 1d point to tingible body macrophages with intracytoplasmic apoptotic bodies. Note the absence of inflammatory cells.

Figure 1e is an image of the thymus from the medullary region of the treated rat in figures 1c and 1d. There are fewer apoptotic cells than in the cortical region due to fewer steroid receptors on the medullary lymphocytes. The arrowhead indicates a single apoptotic lymphocyte whereas the arrow indicates a tingible body macrophage.

Figure 2.

Figure 2a is a transmission electron photomicrograph of normal lymphocytes from the thymus cortex of a control 30-day-old male Sprague–Dawley rat. Figure 2b shows apoptotic lymphocytes from the same region in a rat treated 3 hours previously with 1 mg/kg bodyweight dexamethasone. The lymphocytes are shrunken with irregular shapes and have condensed and peripheralized chromatin. The arrow shows fragmented nuclear material and the arrowhead indicates a portion of cytoplasm that has been extruded. Figure 2c illustrates more advanced lymphocyte apoptosis within the thymus cortex. The apoptotic lymphocytes are smaller with more electron dense condensed nuclear material. The small arrow indicates one of the apoptotic lymphocytes with nuclear fragmentation. The large arrow indicates a tingible body macrophage with intracytoplasmic apoptotic bodies.

Figure 3.

Figure 3a is an image of cortical necrosis in a 30-day-old male Sprague–Dawley rat treated 48 hours previously with 1 mg/kg dexamethasone. The large arrow indicates an enlarged medullary region that is being repopulated with small, mature lymphocytes. The small arrow indicates a region of cortical necrosis and the arrowhead points to the capsule that is expanded by edematous fluid with a few interspersed inflammatory cells. Figures 3b and 3c are higher magnifications of the image in 3a. Figure 3b shows the area of necrosis with a few interspersed apoptotic lymphocytes (arrows). Figure 3c shows the inflammatory cell component which is predominately neutrophils.

Figure 4.

Figures 4a–l are examples of thymuses from a dexamethasone study in 30-day-old male Sprague–Dawley rats. Figure 4a is the normal saline treated control rat thymus. Figures 4b and 4c are high magnifications of the cortical and medullary regions, respectively. Figure 4d is from a rat 24 hours posttreatment. Figures 4e and 4f are high magnifications of the cortical and medullary regions, respectively. Figures 4g and 4j are thymuses from rats 48 hours after treatment. Figures 4h and 4k are high magnifications of the corresponding cortical regions while Figures 4i and 4l are high magnifications of the corresponding medullary regions. When Figures 4a and 4d are compared, a striking tinctorial change is obvious in the treated thymus, especially in the cortical regions due to marked apoptosis of the lymphocytes in this region of the treated rat (Figure 4e). The arrows in Figure 4d indicate regions of tissue tearing, most likely due to lack of cell-to-cell adhesion among the apoptotic cells. The medullary region in figure 4d has increased in area and is less cellular (Figure 4f). This decreased cellularity in the medulla is due to mild lymphocyte apoptosis at 12 hours posttreatment (data not shown). The cortical:medullary ratio has changed significantly in Figure 4d due to a minimal decrease in the cortical area and a mild increase in the medullary region. A qualitative assessment would indicate that the cortical:medullary ratio in Figure 4d is overall approximately 1:2 compared to the normal 2:1 ratio in Figure 4a. Due to tangential cuts through these regions and the generation of different values depending on where the measurement is taken, it is difficult to make a quantitative assessment of this ratio. Although the animals in Figures 4g and 4j were in the same treatment group, there was variability in the tissue response to treatment. Compared

to control (Figure 4a), there is a dramatic decrease in the size of the organ. The thymus in Figure 4g shows recovery of the cortical (Figure 4h) and medullary (Figure 4i) thymic tissue with repopulation of the thymocytes. At low magnification, the overall cortical:medullary ratio is approximately 1:10. The arrow in Figure 4g indicates a parathymic lymph node. The thymus in Figure 4j was more severely affected with necrosis and inflammation of the cortical tissue (Figure 4k). However, there is a robust recovery of the medullary thymocytes evidenced by the darker medullary regions in Figure 4j and the presence of increased numbers of small dark lymphocytes in Figure 4l. The cortical:medullary ratio in Figure 4j is difficult to determine due to the lack of normal cortical tissue. The arrow indicates a parathymic lymph node.

Figure 5.

The arrow in Figure 5a indicates a focal area of increased lymphocytes that are large and pale-staining, with a higher magnification depicted in Figure 5b. An increase in the number of lymphocytes may affect either the cortex or the medulla. These lymphocytes can also be organized into lymphocytic nodules that can resemble germinal centers. Lymphocyte hyperplasia in the thymus with lymphoid follicle formation has not been observed in F344 rats. The formation of actual germinal centers in the thymus of humans and dogs may be an indication of autoimmune disease. Either focal or diffuse lymphoid hyperplasia can also occur after involution of the thymus. Generally, the cortex will show focal accumulations of lymphocytes as well as patchy atrophic changes. Figure 5 photomicrographs are courtesy of Drs. C. Frith and J. Ward.

Figure 6.

These images are from a 2-year-old male B6C3F1 mouse that was treated with phenolphthalein. The arrow in Figure 6a indicates a region of the thymus with a diffuse increase in lymphocyte numbers. These pale lymphocytes appear to diffusely expand the medullary region with no visible cortical tissue remaining. The higher magnifications in Figures 6b and 6c indicate an increase in the number of large, pale lymphocytes, prominent vessels and occasional scattered macrophages with pale golden aggregates of pigmented material (arrows). Figure 6 photomicrographs are courtesy of Drs. Frith and Ward.

Figure 7.

A focal or diffuse increase in thymic lymphocytes may occur in older mice and may involve either the cortex or medulla. This type of lesion usually occurs in thymuses that have undergone physiological involution. In the mouse, it generally occurs in mice older than 6 months of age and is more common in female B6C3F1 mice. In the mouse, lymphocyte hyperplasia is usually characterized by infiltration of the medulla with small, uniform lymphocytes. Figure 7 is an example of diffuse increased lymphocyte numbers in the thymus cortex. The low magnification in Figure 7a illustrates that there is no invasion of the medullary region and the higher magnification in Figure 7b illustrates the small, uniform lymphocytes without a prominent epithelial component. These features help to distinguish this lesion from thymic neoplasias. Figure 7 photomicrographs are courtesy of Drs. Frith and Ward.

Figure 8.

Figure 8a is an example of focal prominent epithelium (arrows) associated with thymic involution in a male 2-year-old F334 rat. This lesion is characterized by prominent tubular structures lined by cuboidal epithelium seen in the high magnification image (Figure 8b). This lesion should be distinguished from thymic neoplasias, such as thymomas, with a prominent epithelial component. Figure 8c and the higher magnification in 8d are images from a 2-year-old male F344 control rat with a focal area of increased epithelial cells at the intersection of two lobules (arrow). Focal areas of increased epithelial cells can be a common finding in the F344 rat.

Figure 9.

The epithelium free areas (EFAs) in the thymus are lymphocyte-rich areas that run from the subcapsular region to deep in the cortex, occasionally bordering the medullary regions. These structures are found in the rat and are considered to be strain-dependent. Special stains are helpful to visualize these regions. Figure 9 is a section of thymus from a control Wistar rat with prominent subcapsular EFAs (arrows). These regions consist of tightly packed CD4/CD8 positive lymphocytes and appear to be lightly encapsulated. These regions would not stain with laminin or keratin, allowing differentiation from the rest of the thymus. Figure 9 photomicrograph is courtesy of Dr. F. Kuper.

Table 1

Checklist for the evaluation of the thymus.

	Yes/↑↓/ Severity grade [*]	No
Cortex		
Increased/Decreased size		
Increased/Decreased number of lymphocytes		
Increased numbers of apoptotic cells		
Increased numbers of tingible body macrophages		
Necrosis		
Medulla		
Increased/Decreased size		
Increased/Decreased number of lymphocytes		
Increased numbers of apoptotic cells		
Increased numbers of tingible body macrophages		
Necrosis		
Prominent epithelial cords and tubules		
Cortex/medulla ratio		
Increased/Decreased		
Epithelium free areas (EFAs) ^{**}		
Not evaluated		
Not present		
Increased/Decreased size		
Increased/Decreased number of lymphocytes		
Increased numbers of apoptotic cells		
Increased numbers of tingible body macrophages		
Necrosis		
Other		
Inflammation		
Cysts		
Pigment		
Extramedullary hematopoiesis (EMH)		

* One recommendation for a grading scheme would be 0 = normal, 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.

** EFAs are most likely species and strain dependent.