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Enhanced Histopathology of Mucosa-Associated Lymphoid Tissue

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Abstract

The secretory epithelial surfaces of the body are a major route of entry for potentially pathogenic substances. The organized mucosal lymphoid tissues that are found within the gastrointestinal and respiratory tracts are therefore particularly important as a first line of defense against harmful compounds. The major function of these mucosa-associated lymphoid tissues (MALT) is to initiate local IgA immune responses, which are then passed on to draining lymph nodes. For enhanced histopathology, the separate compartments of each lymphoid tissue should be evaluated separately for changes in size and lymphocyte cellularity and descriptive rather than interpretive terminology should be used to characterize any changes. The organization of MALT is similar to that of lymph nodes with B-cell-rich follicles and T-cell-rich interfollicular areas. Therefore, these two compartments should be evaluated separately for changes in size and lymphocyte cellularity and the germinal center development within lymphoid follicles should be evaluated as well.

Keywords

MALT; NALT; GALT; BALT; follicles; germinal centers; interfollicular area

Introduction

The mucosa-associated lymphoid tissues (MALT) are dispersed aggregates of nonencapsulated organized lymphoid tissue within the mucosa, which are associated with local immune responses at mucosal surfaces. The three major regions of MALT are the gut-associated lymphoid tissue (GALT), bronchus-associated lymphoid tissue (BALT) and nasal-associated lymphoid tissue (NALT). When oral studies are performed it is advisable to evaluate the GALT and when inhalation studies are performed the BALT and NALT should be evaluated. The organization of the MALT is similar to that of lymph nodes with variable numbers of follicles (B-cell area), interfollicular areas (T-cell area), and efferent lymphatics although afferent lymphatics are lacking. The overlying follicle associated epithelium (FAE) is typically cuboidal with variable numbers of goblet cells and epithelial cells with either microvilli or numerous surface microfolds (M cells). In addition to the organized lymphoid structures, single lymphocytes can be observed within the epithelium, mucosa and lamina propria. All MALTs are morphologically similar although there are location and species differences in the percentage of T and B cells (Haley, 2003). The article by Cesta may be referred to for a more comprehensive review of the normal structure and function of MALT (Cesta, 2006).

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There are special features of stimulated and non-stimulated MALT that should be noted. In the rat, the basal lamina of the NALT and GALT epithelium is often interrupted where there is B and T lymphocyte and macrophage infiltration and should not be confused with ulceration (Kuper et al., 1990). This feature is not typically present in BALT but is reported to occur after intratracheal antigen administration with a concomitant increase in the number of nonciliated cells (Van der Brugge-Gamelkoorn et al., 1986).

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. The evaluation of MALT for enhanced histopathology would include a careful comparison with appropriate controls and an indication of changes in the number and size of follicles and germinal centers and changes in the size and density of the interfollicular area. Other changes to evaluate are hypertrophy of the high endothelial venules (HEV), and the presence, severity and location of apoptotic cells and tingible body macrophages.

Although not strictly a component of enhanced histopathology, other items that can be noted during the evaluation are the location and severity of necrosis, plasma cells, granulocytes, pigmented macrophages, granulomas, etc. An example of a checklist for the changes to be noted in MALT for enhanced histopathology is given in Table 1. This table is intended to be an example of a guideline 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 (Haley et al., 2005).

GALT

The GALT is typically organized into discrete lymphoid aggregates within the mucosa, submucosa and lamina propria of the small intestine called Peyer's patches. These aggregates are typically multiple lymphoid follicles with diffuse lymphatic tissue oriented towards the mucosal side. In the F344 rat, the majority (56%) of lymphocytes in Peyer's patches are B lymphocytes. Examination of all areas of the Peyer's patches can be achieved by evaluating both transverse and longitudinal sections or by using the Swiss roll technique. This technique involves opening 6–8 cm segments of the small intestine in a longitudinal fashion and rolling the tissue with the mucosal side outward along a wooden stick prior to fixation (Figure 1) (Moolenbeek and Ruitenberg, 1981).

Since the intestines are constantly exposed to antigens within the feed material, immunologically stimulated Peyer's patches can be a common finding. There can be a wide range in the amount of antigenic stimulation and resulting tissue changes seen among Peyer's patches within one animal and the size of the Peyer's patch does not always correspond to the amount of antigenic stimulation (Figure 2). There can also be a considerable amount of variation in the amount of antigenic stimulation among animals within a group (treated or untreated). For these reasons the GALT is one of the more challenging MALT tissues to evaluate. It is therefore crucial to perform a careful evaluation of all animals within and between groups to determine if there is a significant difference between treated and control animals. In general, chemicals such as dexamethasone and cyclophosphamide that target other lymphoid organs may also target the gut associated lymphoid tissues, but to a lesser degree (Figure 3).

NALT

In the respiratory tract the NALT is the first site of contact for most airborne antigens since NALT but not BALT appear activated in healthy, untreated, conventionally housed rats (Kuper et al, 1990). There are species differences in the location of NALT within the nasal cavity

(Haley, 2003). In rats the NALT is restricted to the ventral aspects of the lateral walls at the opening of the nasopharyngeal duct (Harkema, 1991) (Figure 4).

NALT is generally present in the third cut cross section (level III) through the head at the region of the middle of the second molar (olfactory region) according to the NTP Histology Specifications (Boorman et al., 1990). At this level there will be extensive ethmoid turbinates on either side of the nasal septum with a large ventrally-located nasopharyngeal duct. After splitting the noses of adult male albino Wistar rats lengthwise into equal halves and histologically evaluating the NALT, Kuper et al. (1990) demonstrated that the NALT extends with its long axis from apical to proximal, with distinct regions of T and B lymphocytes that are not in a fixed location. Therefore, stepwise cross-sections may have to be prepared in order to read all regions optimally.

Since the nasal associated mucosal tissue is the first line of defense against inhaled antigens an increase in the area of lymphoid tissue can be a common finding. However, there is typically a paucity of follicles within the NALT, most likely due to the fast transport of the immunological reaction to the draining cervical lymph nodes. Systemically administered chemicals that result in apoptosis or necrosis of other lymphoid organs may also target the NALT (Figure 5). As with all tissue evaluations, a careful comparison with control animals is needed in order to differentiate background lesions from true treatment-related tissue changes.

BALT

Bronchus-associated lymphoid tissues are organized aggregates of lymphocytes that are located within the bronchial submucosa (Figure 6). These aggregates are randomly distributed along the bronchial tract but are consistently present around the bifurcations of bronchi and bronchioli and always lie between an artery and a bronchus. This tissue can be evaluated with traditional sectioning methods of the lung. These lymphoid aggregates are composed of both B and T cells however, in the F344 rat, the majority (61%) of the lymphocytes are T cells. In various strains of mice (C57Bl/6, BALB/c, and C3D2/F1) the BALT is present but not well developed (Breel et al., 1988). Several types of nonlymphoid cells are also present, including fibroblasts, reticulum cells, macrophages and interfollicular and follicular dendritic cells. Lymphocytes may or may not be organized into lymphoid follicles. Similar to NALT, there is typically a paucity of follicles within the BALT, most likely due to the fast transport of the immunological reaction to the draining regional lymph nodes. In contrast to lymph nodes, BALTs have a relatively high proportion of collagen and reticular fibrils (Figures 7a and 7b). Inhalation studies may result in a local immune response and corresponding histological changes in the bronchus-associated lymphoid tissue and systemically administered chemicals that result in apoptosis or necrosis of other lymphoid organs may also target the BALT (Figures 7c and 7d).

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Figure 1.

This is an illustration of the “Swiss roll technique” for the evaluation of Peyer's patches. This technique involves opening 6–8 cm segments of the small intestine in a longitudinal fashion and rolling the tissue with the mucosal side outward along a wooden stick prior to fixation. Within this segment of small intestine, one region of Peyer's patch is indicated by the arrow.

Figure 2.

The GALT (Peyer's patches) is normally exposed to antigens from the gut lumen. This constant antigenic stimulation results in variable degrees of germinal center development and corresponding lymphocyte apoptosis within the germinal centers. In addition, the size of the Peyer's patch does not always correspond to antigenic stimulation. This normal GALT histomorphological variability exists both within animals and between animals and it is this variability that makes the detection of minor changes in antigenic stimulation difficult to discern between control and treated animals within a study. The Peyer's patches in Figure 2A–2P are all from the same control Sprague–Dawley rat and illustrate this concept. The Peyer's patch that is shown at increasing magnifications in Figures 2A–2C is relatively small and quiescent. The Peyer's patches in Figure 2D are similar in size to that in Figure 2A, however the germinal centers are paler staining and contain more apoptotic bodies (Figures 2E–2F), indicating an increased level of antigenic stimulation. The Peyer's patches in Figure 2G are robust, are larger than those in Figures 2A and 2D, and are easily seen on gross examination; however they are immunologically quiescent, lacking germinal centers (Figures 2H–2I). The Peyer's patches in Figure 2J are not much larger than those in Figure 2G, however they contain a large and small secondary follicle (long and short black arrows, respectively) with pale staining germinal centers (Figure 2K). Within the germinal centers there is a mild degree of lymphocyte apoptosis indicated by scattered hyperchromatic cellular debris and tingible body macrophages (Figure 2L, arrow). The Peyer's patches in Figure 2M are similar in size to those in Figure 2J and more than twice the size of those in Figure 2D, however the germinal centers

are darker staining (fewer lymphoblasts) (Figure 2N, arrow) and there is minimal lymphocyte apoptosis (Figures 2O–2P, arrows).

Figure 3.

Peyer's patches from control (left panel, Figures 3A, 3C, 3E, 3G) and dexamethasone treated (right panel, Figures 3B, 3D, 3F, 3H) Sprague–Dawley rats are shown from low magnification (top) to high magnification (bottom) illustrating a treatment related change. The Peyer's patch from the control rat (left panel) is immunologically quiescent with a predominance of small mature lymphocytes. The Peyer's patch from the treated rat (right panel) is characterized by a prominent secondary follicle with an eosinophilic germinal center (arrow, Figure 3B) surrounded by scattered necrotic eosinophilic debris (arrowhead, Figure 3B). The germinal center contains many apoptotic cells with scattered tingible body macrophages (arrow, Figure 3H) and large paler-staining lymphoblasts.

Figure 4.

This is a section at level III of the nasal passage of a control Sprague–Dawley rat with prominent ethmoid turbinates and a nasopharyngeal duct (P). The two aggregates of nasal-associated lymphoid tissue typically lie at the rostrolateral aspect of this duct (arrowheads).

Figure 5.

Figure 5A illustrates NALT from a control Sprague–Dawley rat. Figure 5B is the NALT from a Sprague–Dawley rat that was treated with dexamethasone 12 hours earlier and illustrates a minimal increase in lymphocyte apoptosis (arrows) when compared to controls. Figure 5C depicts NALT from another rat, treated with dexamethasone 48 hours earlier, with a mild increase in apoptotic lymphocytes and early loss of cells (arrow).

Figure 6.

Figures 6A and 6B illustrate the random submucosal location of BALT (arrows) within the bronchial wall of a control Sprague-Dawley rat; however they are always situated between an artery and a bronchus.

Figure 7.

Figures 7A and 7B are low and high magnifications of normal rat BALT. This example lacks a lymphoid follicle and is composed of a predominance of small mature lymphocytes with interspersed collagen and reticular fibrils. Figures 7C and 7D are low and high magnifications of BALT from a treated rat. There is an overall decrease in the area and cellularity of lymphoid tissue and there are scattered apoptotic lymphocytes indicated by the arrows in Figures 7C and 7D. In normal BALT, apoptotic cells are typically confined to the follicular areas, when present.

Table 1

MALT: specify type (e.g., GALT, BALT, NALT).

	Yes/↑↓/Severity grade [*]	No
Follicles		
Increased/Decreased follicle number		
Increased/Decreased follicle size		
Increased/Decreased numbers of lymphocytes		
Increased/Decreased germinal center number		
Increased/Decreased germinal center size		
Interfollicular area		
Increased/Decreased size		
Increased/Decreased numbers of lymphocytes		
Increased numbers of		
Apoptotic cells		
Tingible body macrophages		
Plasma cells		
Pigmented macrophages		
Granulocytes (type)		
Prominent HEV		
Granuloma/macrophage aggregates		
F&A ulceration		
Necrosis (location)		
Other		

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