Distribution of Lymphatics in Human Palatine Tonsils: 
A Study by Enzyme-Histochemistry and Scanning Electron 
Microscopy of Lymphatic Corrosion Casts

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Summary. The distribution of lymphatics in human palatine tonsils was studied by enzyme-histochemistry for 5'-nucleotidase (5'-Nase) and scanning electron microscopy (SEM) of lymphatic corrosion casts. The palatine tonsils were found to possess lymphatics in the parafollicular area (i.e., interfollicular, interfolliculo-septal, and folliculo-septal area), in the connective tissue septa, and in the capsules, but not in the subepithelial area between the follicles and the follicle-associated epithelia or within the follicles. The tubular lymphatics originated some 200–300 µm below the epithelium and formed a three-dimensional network in the parafollicular area. Some lymphatics around the lower part of the follicle were flat, wide, and irregular in shape, and thus appeared to be lymphatic sinuses, referred to as perifollicular lymphatic sinuses. The lymphatics in the parafollicular area drained into the septal lymphatics, which ran rather straight in the connective tissue septa. The septal lymphatics finally gathered into the broader capsular lymphatics. Most of the septal and capsular lymphatics were endowed with valves. Our results indicate that lymphocytes and fluid from the follicles and the subepithelial region enter the perifollicular lymphatic sinuses and/or the interfollicular lymphatics, pass through the interfolliculo- and folliculo-septal lymphatics, and finally enter the septal and capsular lymphatics to leave the tonsil.

Human palatine tonsils are located in the fauces, i.e., in the front line of mucosa-associated lymphoid tissues. Being thus strategically placed for protection of the body against invasion by bacteria and foreign proteins, they must readily uptake and recognize antigens and start appropriate immune reactions (OLÁH, 1978; OWEN and NEMANIC, 1978). The recirculation (homing) of lymphocytes is indispensable to effective reactions. The generally accepted model for the way lymphocytes pass from the blood into the lymph within the tonsils, the appendices, and Peyer’s patches is via post-capillary venules with a high-walled endothelium, called high-endothelial venules (HEVs), and then a return to the blood via lymphatics (GOWANS and KNIGHT, 1964; FORD, 1975). In this way, HEVs and lymphatics in those secondary lymphoid tissues serve as the route for the recirculation of lymphocytes during immune reactions.

The three-dimensional arrangement of the blood vascular system of palatine tonsils has been demonstrated for humans (OHTANI et al., 1989) and for rabbits (TERASAWA et al., 1988). UMETANI (1977) revealed by scanning electron microscopy (SEM) that, in most cases, lymphocytes passed intracellularly through the endothelial cells of the HEVs in the rabbit tonsil. In his conventional light microscopy of the palatine tonsil in the rabbit, FIORETTI (1961) described four types of lymphatics: subepithelial lymphatics, centronodular lymphatics, perinodular lymphatic sinuses, and the internodular and internodulo-capsular lymphatic network. Our routine microscopy in this study, however, did not show any centronodular or subepithelial lymphatics in the palatine tonsil. Furthermore, our previous studies demonstrated that the appendix and Peyer’s patches in the rabbit possess well-developed perifollicular lymphatic sinuses and interfollicular lymphatics, but no lymphatics were recognized within the follicles or in the subepithelial region between the follicles and the follicle-associated epithelia. These findings led us to re-examine the distribution of lymphatics in the palatine tonsil by enzyme-histochemistry for 5’-nucleotidase (WACHSTEIN and MEISEL, 1957), which allowed a clearer identification of lymphatics (VETTER, 1970;
WERNER et al., 1987) than previously employed methods. The three-dimensional arrangement of the lymphatics of the tonsil was studied by SEM of lymphatic corrosion casts.

MATERIALS AND METHODS

Human palatine tonsils were obtained from 14 adults and 20 children undergoing routine tonsillectomies. In all cases the pre-operative diagnosis was chronic tonsillitis.

Light microscopy

The tonsils were washed out by physiological salt solution, sliced into several pieces and then immersed for 2 h in a fixative which consisted of 6% paraformaldehyde-1% CaCl₂ in 0.1 M cacodylate buffer (pH 7.2) containing 7% sucrose. Some sections, 40 μm in thickness, were processed in a cryostat, and others, 200 μm in thickness, by a vibratome (Vibratome Series 1000, Lancer). In some tonsils, the capsular region was removed from the tonsils by scissors. All the sections and the capsular preparations were then incubated for 30 min at 37°C in a lead-based standard medium for 5'-Nase reaction according to the method by WACHSTEIN and MEISEL (1957). Then the sections were rinsed in distilled water and immersed in 1% yellow ammonium sulfide solution for 2 min. After the distilled water rinsing, some sections were further treated by the azo-dye method (BURSTONE, 1962) for 60 min to visualize alkaline phosphatase (ALPase). Finally, all of the treated specimens were thoroughly rinsed in distilled water, mounted in glycerin, and observed under a light microscope.

Scanning electron microscopy of lymphatic corrosion casts

The lymphatic corrosion casts were made according to the method described by OHTANI (OHTANI and OHTSUKA, 1985; OHTANI et al., 1986; OHTANI, 1987; OHTANI and MURAKAMI, 1990, 1992a). Approximately 2-3 ml of Mercox CL-2B-5 (Dainippon Ink., Tokyo), which was diluted to about 40% (vol/vol) with monomeric methyl methacrylate, was puncture-injected intraparenchymally. A syringe (6 ml) with a 23 gauge needle was used for injection. The injection pressure was controlled by hand while observing the injected medium filling the lymphatics in the tonsils. The injected tissues were put in hot water (60°C) for several hours, and then tissue elements were dissolved in a 15% NaOH solution overnight. The corrosion casts obtained by this method were washed gently in running water. They were air-dried, mounted on aluminum stubs with silver paste, coated with gold, and observed in a Hitachi S-4500 SEM with an accelerating voltage of 10–15 kV.

RESULTS

Light microscopy

After incubation in the standard medium for 5'-Nase, the lymphatics were stained with brown dye (Figs. 1a, 2-5) while the blood vessels were stained only slightly. Treatment by the azo-dye method visualised the arterial capillaries and arterioles in dark blue, but the venous capillaries and venules were not stained at all (Figs. 1b, 6). The stained lymphatics were distributed in the parafollicular area (including the interfollicular, interfolliculo-septal, and folliculo-septal areas), and within the septa and capsules (Figs. 1-6). There were no lymphatics in the subepithelial region, i.e., between the follicles and the follicle-associated epithelia (Figs. 2, 5) or within the follicle (Figs. 1-3). The tubular lymphatics originated some 200 to 300 μm below the epithelium in the parafollicular area (Fig. 2). The lymphatics surrounding the follicles showed wide, irregular lumens (Figs. 1, 5). In the 200 μm thick specimens, the lymphatics in the parafollicular area repeatedly fused and divided, forming a network around the follicles and gradually gathering into the broader lymphatic sinuses around the lower

Fig. 1. Light micrographs of the human palatine tonsil reacted to 5'-Nase (a) and to 5'-Nase and ALPase (b): cryostat sections (40 μm thick). Dark brown staining indicates a positive reaction for 5'-Nase activity in the lymphatics, while dark blue staining shows that for ALPase in the arterial capillaries and arterioles. F lymph follicle, s perifollicular lymphatic sinus. ×140

Fig. 2. A light micrograph of the human palatine tonsil reacted to 5'-Nase: cryostat section (40 μm thick). The tubular lymphatics (arrowheads) originate some distance below the epithelium (E), partially surrounding the lymph follicle (F). They drain into the interfolliculo- and folliculo-septal lymphatics, which in turn gather into the septal lymphatics (arrows). No lymphatics can be seen in the subepithelial region. ×70

Fig. 3. A light micrograph of the human palatine tonsil reacted to 5'-Nase: cryostat section (40 μm thick). The lymphatics in the septum (S) collect lymphatics in the parafollicular area and take relatively straight courses through the connective tissue septum. ×70
Figs. 1-3. Legends on the opposite page.
part of the follicles (Fig. 5). The lymphatics in the connective tissue septa took rather straight courses (Figs. 2, 3), with most of them possessing valves. The capsular lymphatics were broad and equipped with distinctive valves (Fig. 4); however, they were not endowed with smooth muscle cells.

**SEM of lymphatic corrosion casts**

SEM observation of the corrosion casts of the tonsils clearly showed the three-dimensional structure of the lymphatic network comprising lymphatics of various sizes and shapes (Figs. 7, 8). The lymphatics in the parafollicular area were mostly tubular in shape. They repeatedly fused and divided, forming a three-dimensional network in the interfollicular, interfolliculo-septal and folliculo-septal areas (Fig. 7). The lymphatics surrounding the follicles were flat, wide, and irregular in shape; thus, they appeared to be lymphatic sinuses, to be referred to as perifollicular lymphatic sinuses (Fig. 7). These perifollicular lymphatic sinuses were especially well developed around the lower portion of the follicle (Fig. 7). The sinuses, however, were not continuous along the entire wall of the lymph follicle and, in fact, had many interruptions. There were no replications of lymphatics within the dome and the follicle; instead, scrambled egg-like structures were frequently observed, presumably representing the casts of tissue spaces of the follicles. The lymphatics in the connective tissue septum, gathering parafollicular lymphatics *en route*, ran quite straight through the septum, and then drained into the capsular lymphatics. The capsular lymphatics were broader than those in the parafollicular area or in the septa. Most of the septal and capsular lymphatic corrosion casts showed notches indicative of valve locations (Fig. 8).

**Fig. 4.** A light micrograph of the human palatine tonsil reacted to 5'-Nase. The capsular lymphatic vessel is broader and endowed with valves (arrows). A section obtained by a vibratome (200 μm thick). ×40

**Fig. 5.** A light micrograph of the human palatine tonsil reacted to 5'-Nase. The lymphatic sinuses form a network around the lower portion of the follicles: vibratome section (200 μm thick). C crypt, E epithelium. ×140

**Fig. 6.** A light micrograph of the human palatine tonsil reacted to 5'-Nase and ALPase. The interfollicular area shows that ALPase of the arterial capillaries and arterioles are stained in dark blue, and 5'-Nase of the lymphatics in dark brown. ×70
Fig. 7. A scanning electron micrograph of the lymphatic corrosion cast of the human palatine tonsil. The tubular lymphatics, forming a network in the parafollicular area (P), are gathered into the broader lymphatic sinuses (s) surrounding the lower part of the follicle (F). ×110

Fig. 8. A scanning electron micrograph of the lymphatic corrosion cast (L) in the capsular region of the human palatine tonsil showing distinctive notches (arrowheads) indicative of valve-locations. ×75
DISCUSSION

Using enzyme-histochemistry for 5'-Nase and SEM of lymphatic corrosion casts, the present study has demonstrated the three-dimensional organization of the lymphatic network of human palatine tonsils. As has been established in other organs (Vetter, 1970; Werner et al., 1987; Kato and Miyauchi, 1989; Kato, 1990), this enzyme-histochemistry permits the clear identification of lymphatics in the human palatine tonsil as well. Double staining for 5'-Nase and ALPase (Kato and Miyauchi, 1989; Kato, 1990) has facilitated the distinction of lymphatics from blood vessels. The corrosion casts produced in our present study are taken to represent the lymphatic lumens, since the SEM images of the casts correspond well to the light microscopic images of enzyme-histochemically stained lymphatics. The injected casting medium frequently fills the tissue spaces as well, but these spaces can be easily distinguished from the lymphatic casts. However, there remains a possibility...
that some lymphatics may not be filled with the casting medium.

This study has shown that, in human palatine tonsils, lymphatics exist in the interfollicular, interfolliculo-septal and folliculo-septal areas, and within the septa and capsules. The lymphatic pattern of the human palatine tonsil revealed by the present study is schematically illustrated in Figure 9. In contrast with the report by FIORETTI (1961), there are no lymphatics within the follicles. There are no lymphatics in the subepithelial regions between the follicles and follicle-associated epithelia. The distribution pattern of the lymphatics revealed in the present study indicates that lymphocytes and fluid from the follicles and subepithelial regions enter the perifollicular lymphatic sinuses and/or interfollicular lymphatic, pass through the interfolliculo- and folliculo-septal lymphatics, and finally successively enter the septal and capsular lymphatics to leave the tonsil. The organization of the lymphatics in the human palatine tonsil basically conforms to that of other secondary lymphoid tissues such as Peyer's patches, appendices, and the sacculus rotundus in the rabbit (OHTANI et al., 1986; OHTANI and MURAKAMI, 1990, 1992b), although there are some minor differences which will be discussed below.

The perifollicular lymphatic sinuses described by FIORETTI (1961) in the rabbit tonsil have been confirmed to exist also in the human palatine tonsil. In addition, the present study has revealed their three-dimensional arrangement. The voluminous perifollicular lymphatic sinuses of both appendices, Peyer's patches, and sacculus rotundus in the rabbit appear to have a great potential capacity as reservoirs and drainage routes for fluid and lymphocytes (BOCKMAN, 1983; OHTANI et al., 1986; OHTANI and MURAKAMI, 1990, 1992b). The perifollicular sinuses in the human tonsil were, however, less developed than those in other gut-associated lymphoid tissues (GALT) such as Peyer's patches, appendices, and the sacculus rotundus in the rabbit (OHTANI et al., 1986; OHTANI and MURAKAMI, 1990, 1992b), although it was indicated that the possession of valves is not the sufficient condition for collecting lymphatics. More correlative studies of functions and morphology of the lymphatics seem necessary to define the distinction between initial and collecting lymphatics.

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**REFERENCES**


