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Blood microvascular organization of the nasal-associated lymphoid tissue of the guinea pig: a scanning electron microscopic study of corrosion casts.

Satoko Okada* Aiji Ohtsuka[†] Hirofumi Akagi[‡]
Kazunori Nishizaki** Yu Masuda^{††}

*Okayama University,

[†]Okayama University,

[‡]Okayama University,

**Okayama University,

^{††}Okayama University,

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Satoko Okada, Aiji Ohtsuka, Hirofumi Akagi, Kazunori Nishizaki, and Yu Masuda

Abstract

It has previously been confirmed that the guinea pig has aggregations of 10-20 lymphoid follicles at the junction of the nasal cavity and the nasopharyngeal duct. The vascular architecture of this nasal-associated lymphoid tissue (NALT) was studied by the corrosion cast/scanning electron microscope method. The NALT was supplied by branches of the inferior nasal artery. These afferent arterial branches gave off arterioles to the follicles and the interfollicular regions, where the arterioles ramified into capillaries. Some of these arterioles reached the subepithelial region to form a single-layer dense capillary network. The subepithelial capillaries gathered into short collecting venules, which in turn drained into high endothelial venules (HEV) in the interfollicular region. The HEV, which also receives tributaries from the follicular and interfollicular capillary plexuses, descended in the interfollicular regions and finally flowed into the efferent veins at the bottom of the NALT. Indentations impressed by high endothelial cells (HEC) were prominent on the surface of the HEV casts, and their frequency was larger in the upper course or segments than in the lower. This suggests that the incidence of HEC in the upper segments is higher than in the lower segments, and these findings are consistent with the hypothesis that some substances which are taken up into the subepithelial capillaries and transported to the venules induce differentiation and maintain of HEVs.

KEYWORDS: nasal-associated lymphoid tissue, vascular corrosion cast, microvascular architecture, high endothelial venule, guinea pig

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Blood Microvascular Organization of the Nasal-Associated Lymphoid Tissue of the Guinea Pig: A Scanning Electron Microscopic Study of Corrosion Casts

Satoko OKADA, Aiji OHTSUKA^a, Hirofumi AKAGI, Kazunori NISHIZAKI and Yu MASUDA

Departments of Otorhinolaryngology and Anatomy^a, Okayama University Medical School, Okayama 700, Japan

It has previously been confirmed that the guinea pig has aggregations of 10-20 lymphoid follicles at the junction of the nasal cavity and the nasopharyngeal duct. The vascular architecture of this nasal-associated lymphoid tissue (NALT) was studied by the corrosion cast/scanning electron microscope method. The NALT was supplied by branches of the inferior nasal artery. These afferent arterial branches gave off arterioles to the follicles and the interfollicular regions, where the arterioles ramified into capillaries. Some of these arterioles reached the subepithelial region to form a single-layer dense capillary network. The subepithelial capillaries gathered into short collecting venules, which in turn drained into high endothelial venules (HEV) in the interfollicular region. The HEV, which also receives tributaries from the follicular and interfollicular capillary plexuses, descended in the interfollicular regions and finally flowed into the efferent veins at the bottom of the NALT. Indentations impressed by high endothelial cells (HEC) were prominent on the surface of the HEV casts, and their frequency was larger in the upper course or segments than in the lower. This suggests that the incidence of HEC in the upper segments is higher than in the lower segments, and these findings are consistent with the hypothesis that some substances which are taken up into the subepithelial capillaries and transported to the venules induce differentiation and maintain of HEVs.

Key words: nasal-associated lymphoid tissue, vascular corrosion cast, microvascular architecture, high endothelial venule, guinea pig

The oronasopharynx is a front and strategic site for the first exposure to inhaled bacteria, viruses and other exogenous antigens. In the human pharynx, the tonsils arrange in a circular manner to form Waldeyer's ring. In rodents including rats (1), mice (2) and hamsters (3), lymphoid follicles aggregate at the ventrolateral wall of the rostral entrance of the nasopharyngeal duct. This follicle aggregation has been called the nasal-associated lymphoid tissue (NALT), and has been used as an experimental equivalent of Waldeyer's ring for studying the nasopharyngeal mucosal immune system (4, 5). However, as far as we know, there are no reports on whether or not guinea pigs have such lymphoid tissue in the nasopharyngeal region.

High endothelial venules (HEV) have a specialized endothelium through which lymphocytes migrate into lymphoid follicles in the secondary lymphoid organs, such as peripheral lymph nodes, mucosa-associated lymphoid tissues (Peyer's patches, appendix, tonsils and NALT) (6, 7). The vascular organization of these lymphoid organs is important for their functional histoarchitecture (8). The microvascular architecture of the rat NALT has been demonstrated using scanning electron microscopy of corrosion casts (9). The aim of the present study was to investigate whether the guinea pig has NALT at the junction of the nasal cavity and the nasopharyngeal duct, and to evaluate the blood microvascular arrangement by scanning electron microscopy of corrosion casts (10).

Materials and Methods

Animals. Ten female Hartley guinea pigs weighing 450-750 g were used. These animals were raised in a conventional laboratory room and given food and water

* To whom correspondence should be addressed.

freely.

Scanning electron microscopy. Eight animals were used under deep anesthesia with diethyl ether. The thoracic cavity was opened, the thoracic aorta was ligated, and about 100 ml of 0.9 % NaCl solution was infused through the cannulated ascending aorta. Subsequently, 30–40 ml of commercially available casting resin (Mercor, Oken Shoji, Tokyo, Japan) was injected. In some cases, 50 ml of 10 % formalin in physiological saline was perfused prior to the resin injection. The facial region, including the nose and nasopharynx, was soaked in hot water at 50–60 °C for 2–4 h, then corroded in a hot (60 °C) 15–20 % NaOH solution overnight, and washed in running tap water.

The corrosion casts were frozen, cut into appropriate blocks, dried, and mounted on metal stubs using silver paste. After coating with gold, the casts were observed with a scanning electron microscope (S-2300, Hitachi) at an accelerating voltage of 5 kV.

Light microscopy. The remaining two animals were used under deep ether anesthesia. After perfusion-fixation with 4 % paraformaldehyde in 0.1 M phosphate buffer (pH 7.4), nasopharyngeal tissues including the NALT were excised and embedded in paraffin. Sections of the NALT were stained with hematoxylin and eosin.

Results

Light microscopy. In the guinea pig, as in the rat, 10–20 lymphoid follicles aggregated at the ventrolateral floor of the junction of the nasal cavity and the nasopharyngeal duct, to form the NALT (Fig. 1). Each follicle had a well-developed germinal center (Fig. 2A). The dome epithelium covering the follicle showed a slight protrusion, and consisted of non-ciliated columnar cells among which many lymphocytes infiltrated, so that so-called lympho-epithelial symbiosis was formed (Fig. 2B).

HEV, whose endothelial cells were cuboidal, were seen in the parafollicular regions but not within the follicles (Fig. 2C). Among HEC, many lymphocytes were observed.

Scanning electron microscopy of vascular casts. The mucosa of the nasopharyngeal junction, including the NALT, was supplied by branches of the inferior nasal artery which was derived from the sphenopalatine artery. The sphenopalatine artery had three branches in the nasopharyngeal region: the superior pharyngeal artery (SPA), the anterior nasal septal artery

(ANSA) and the inferior nasal artery (INA). The SPA supplied the caudal half of the nasopharyngeal duct. The ANSA supplied the nasal septum. And the INA supplied the rostral half of the nasopharyngeal duct, including the NALT. At the bottom of the NALT, the inferior nasal artery gave off a nutrient branch to the palatine bone and arterial branches to the NALT (NALT arteries). The NALT arteries issued thick afferent arterioles to the NALT and thin periosteal arterioles. The latter gave rise to a coarse capillary plexus in the periosteal layer between the palatine bone and the NALT. (Fig. 3)

The afferent arterioles entered the follicles and the interfollicular regions, and ascended toward the luminal aspect, giving off many capillaries *en route*. The capillaries repeatedly branched and anastomosed to form coarse follicular and interfollicular capillary plexuses in respective regions. Some of the afferent arterioles of the NALT reached the subepithelial region, where they gave rise to the subepithelial or dome capillary network (Fig. 4). The entire dome capillary network overlying the follicle protruded slightly (Fig. 5). The mesh size of the network covering the follicle was larger than that of the ordinary nasopharyngeal mucosa.

The subepithelial capillaries gathered into short collecting venules which originated just beneath the epithelial layer (Fig. 4). Then, the collecting venules anastomosed with other collecting venules to form thick venules in the interfollicular region. Some capillaries seemed to flow directly into these thick venules. The vascular casts of the

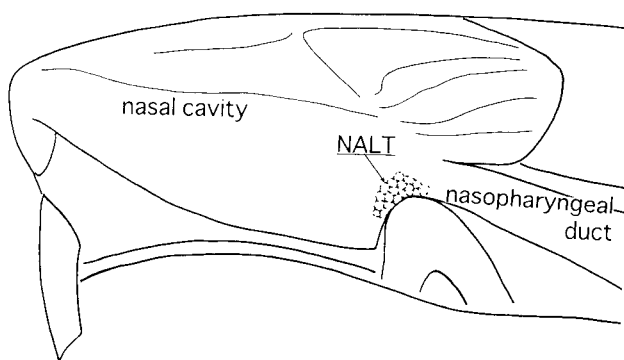


Fig. 1 A schematic presentation of the guinea pig's nasopharyngeal region to show the location of the nasal-associated lymphoid tissue (NALT).

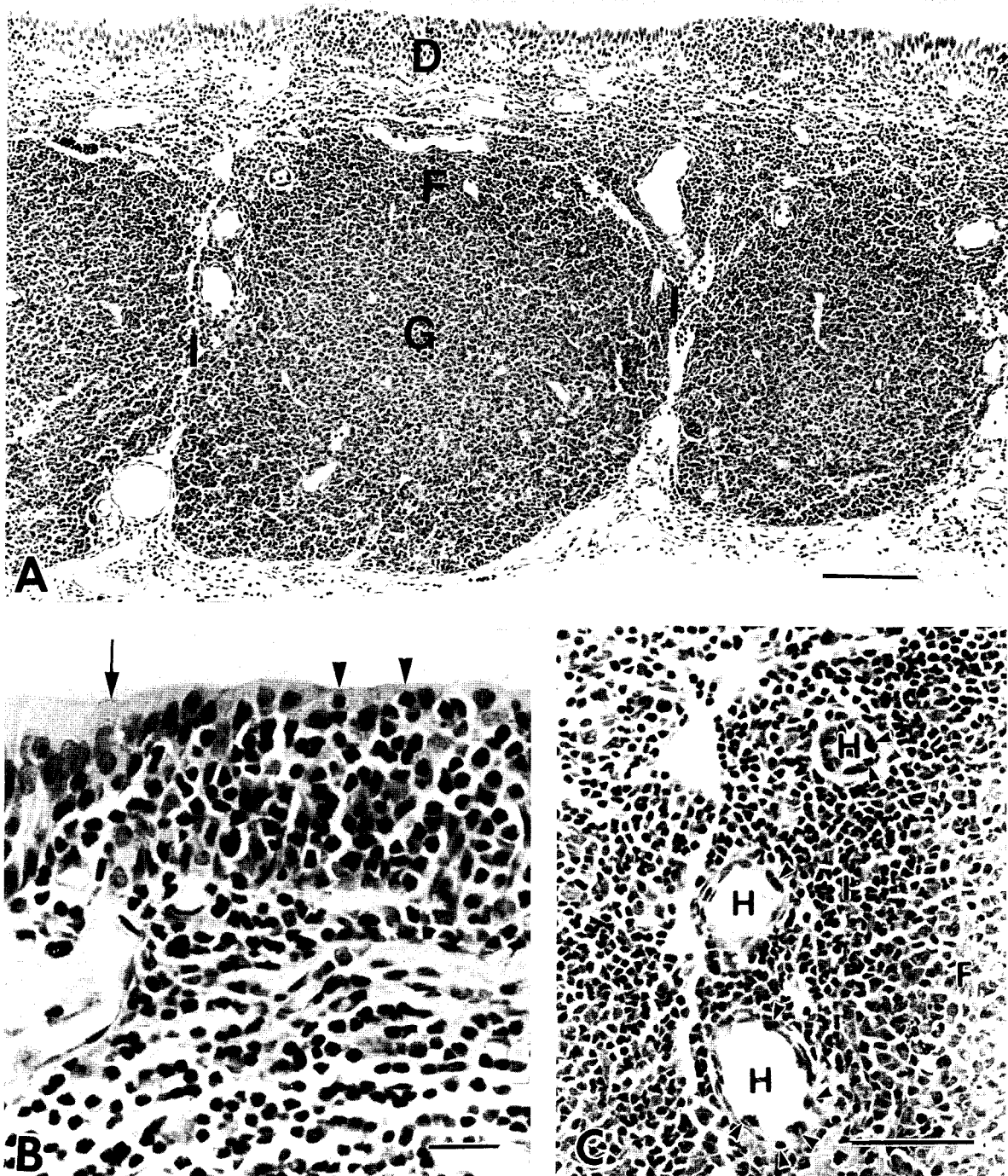
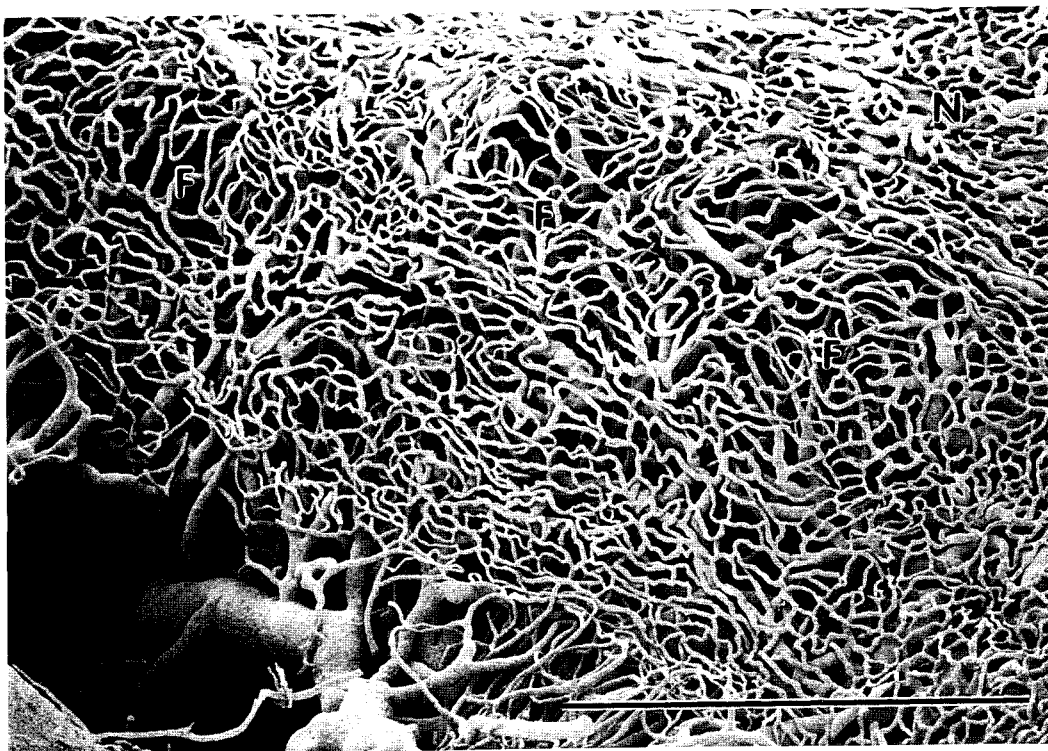
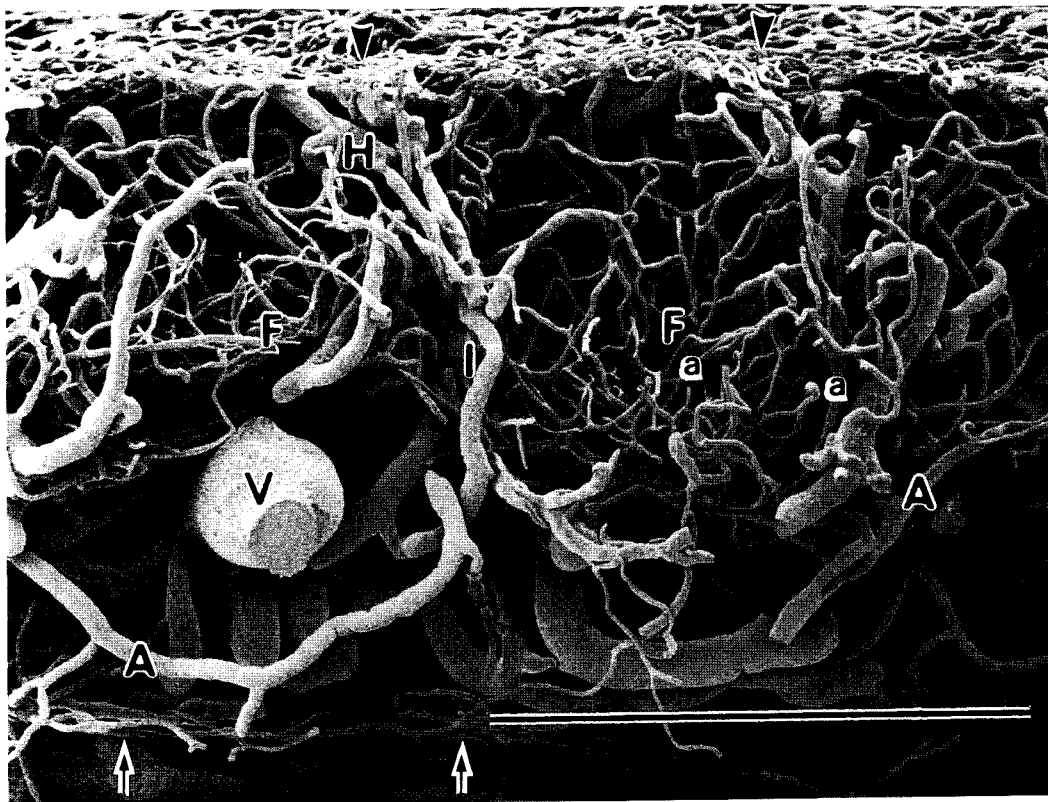


Fig. 2 Light micrographs of the perfusion-fixed guinea pig NALT stained with hematoxylin and eosin.

A: Lymphoid follicles (F) with well-developed germinal centers (G) aggregate below the epithelium of the nasopharyngeal duct. D: dome area (Bar = 100 μ m) **B:** High-power view of the dome epithelial region. Many lymphocytes (arrowheads) infiltrate among the epithelial cells (arrow). (Bar = 20 μ m) **C:** The high endothelial venules (H) in the interfollicular region (I). Many lymphocytes (arrowheads) are migrating through the endothelial cells. (Bar = 50 μ m) NALT: See Fig. 1.



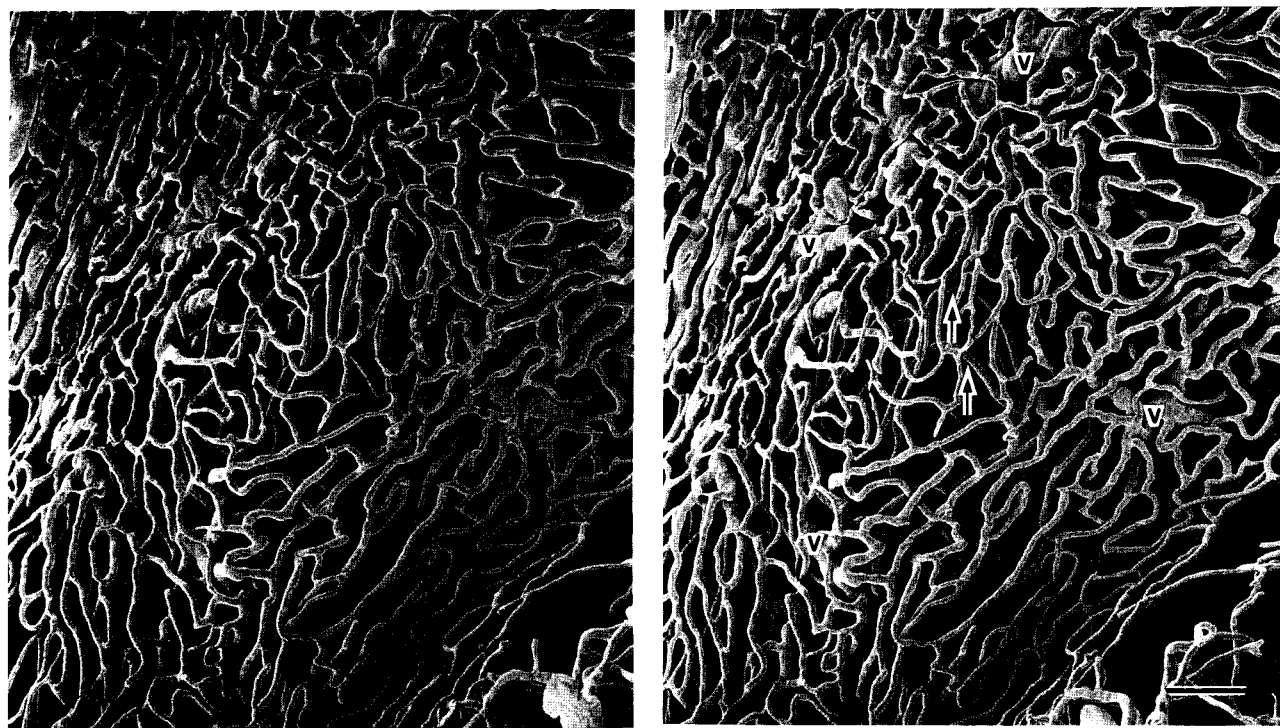


Fig. 5 Stereo-paired micrographs (wall-eye view) of the luminal aspect of the cast NALT. Arterioles (arrow) and venules (v) supply the subepithelial capillary network. Note the dome subepithelial network slightly protrudes to the luminal side. (Bar = 100 μ m). NALT: See Fig. 1.

thick venules had irregular indentations on their surfaces (Fig. 6). From these indentations, these venules were identified to be HEV (8). Such indentations were clearly observed in the casts of the specimen fixed with formalin prior to the resin injection. The frequency of the indentations was larger in the upper than in the lower course or segments (Fig. 6).

HEV drained the follicular and interfollicular capillary plexuses. Then, they descended in the interfollicular region, and flowed into the efferent veins at the bottom of the NALT. These efferent veins received venules from the periosteal capillary plexus, and finally left the NALT.

Discussion

The upper respiratory tract is a first line defense against inhaled exogenous antigens. Lymphoid tissues in the nasal and pharyngeal regions are strategically important. In rodents including rats (1), mice (2) and hamsters (3), the NALT is well developed at the junction of the nasal cavity and the nasopharyngeal duct. As far as we know, there are no reports on whether or not the guinea pig has NALT. The present results confirm that the guinea pig has distinct and well-developed NALT. The location and histological constitution of the guinea pig

Fig. 3 A scanning electron micrograph of the vascular corrosion cast of the guinea pig NALT. At the bottom of the NALT, branches (A) of the NALT artery give off arterioles (a) to the follicles (F) and the interfollicular regions (I), where they give rise to coarse capillary plexuses. Some of the arterioles reach the subepithelial region where they make a dense capillary network (arrowheads). arrows: periosteal capillaries V: veins; H: high endothelial venules (Bar = 1 mm). NALT: See Fig. 1.

Fig. 4 Luminal view of the vascular cast of the NALT. Mesh size of the capillary network covering the follicle (F) is larger than that of the ordinary nasopharyngeal mucosa (N). (Bar = 1 mm). NALT: See Fig. 1.

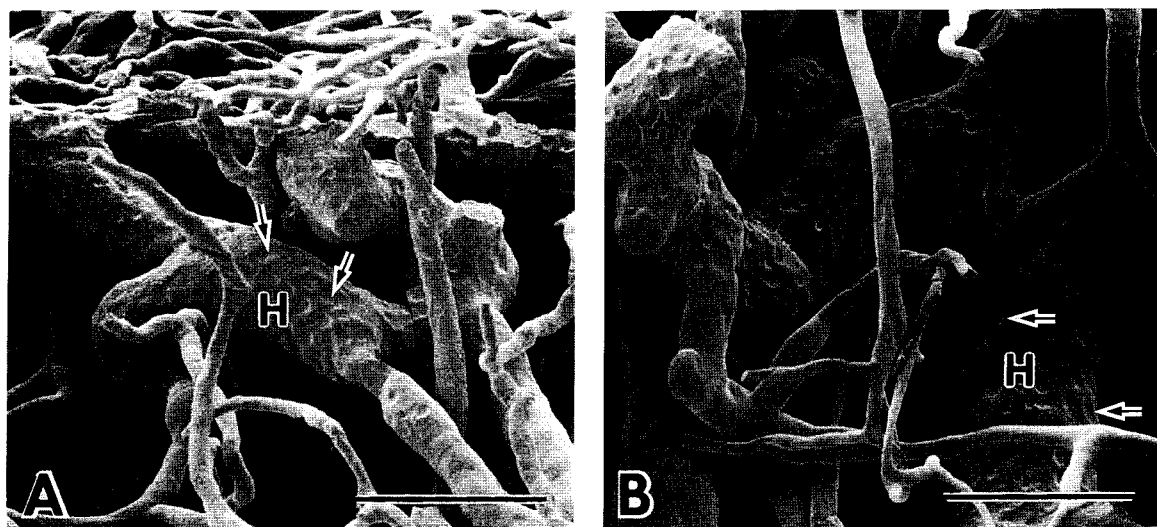


Fig. 6 Highly magnified casts of high endothelial venules (H). **A:** In the upper course or initial segments, indentations on the HEV casts (arrows) are large (high). (Bar = 100 μ m). **B:** In the lower segments, the frequency of indentations (arrows) is low. (Bar = 100 μ m). HEV: high endothelial venules.

NALT was similar to that of rats (4, 9) and mice (11).

In the present scanning microscopic study, the microvasculature of the guinea pig NALT was confirmed to be similar to that of the rat (9). In the human and rabbit palatine tonsils, the capillaries immediately below the crypt epithelium were large and contained many bulges, and there were many switch-back loops of capillaries below the epithelium (12, 13). In the guinea pig NALT, as observed in the rat NALT (9), the dome capillary network is nearly flat and shows no such switch-back loops. As suggested by Hayashi *et al.* (9), this is caused by the difference of the epithelium covering the lymphoid follicles: that is, the palatine tonsil is covered with the stratified squamous epithelium with small papillae, whereas the NALT with columnar epithelium does not have papillae.

In the interfollicular region, there were thick venules whose casts had many irregular concavities or indentations on their surfaces. The cast concavities indicate the HEC projecting into the lumens (8).

It is noteworthy that the HEVs are downstream of the sinusoidal capillaries which are located immediately beneath the dome-epithelium into which many lymphocytes infiltrated. This microvascular pattern is more or less the same as that in other mucosa associated lymphoid tissues, such as tonsils (12) and Peyer's

patches (14). As suggested by Ohtani *et al.* (12), this microvascular connection seems to facilitate the uptake of some substances into the subepithelial capillaries and their transport to the postcapillary venules where their concentrations can reach above a certain level so that the specialized endothelial cells of HEVs may be induced by such local microenvironmental factors. Some experimental and pathological studies have suggested the existence of such factors: HEVs can occur in association with chronic inflammation (15-18); interleukin-1 enhances the functional capacity of cultured human umbilical vein endothelium to bind leukocytes (19, 20); interferon- γ can induce the expression of an antigen specific for endothelial cells involved in lymphocyte traffic (21); the HEVs in the rat lymph nodes disappear within 3 weeks after occlusion of afferent lymphatics but reappear following antigen injection into the nodes (22).

It has been reported that the surface of endothelial cells of HEVs appears as a proximal segment with a cobblestone surface pattern and a distal segment of interlacing cytoplasmic plates (23). Such differences in HEV may correspond with our observation that indentations on HEV cast were more frequent in the upper course than in the lower course. This suggests that HECs in the upper or initial segment occur more frequently and distinctly than in the lower. Such differences in the occurrence of

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HECs may be explained by a gradation of HEC-inductive substances, and is consistent with the hypothesis (12) that some substances which are taken up into the subepithelial capillaries and transported to the venules induce differentiation and maintenance of HEVs.

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