Developmental Study of the Anal Tonsil in the Laboratory Shrew, *Suncus murinus**

Kazuyoshi SAKAI¹, Ai TANAKA² and Genzoh ISOMURA¹

Department of Anatomy¹, Fujita-Health University, College, Toyoake; and School of Health Science², Faculty of Medicine, Kanazawa University, Kanazawa, Japan

Received October 5, 2001; revised December 6, 2001

Summary. The laboratory shrew, *Suncus murinus*, which lacks such gut associated lymph organs as the appendix and Peyer's plates, was recently demonstrated (KUBO and ISOMURA, 1996) to possess a pair of anal tonsils at the end of its rectum. The present paper deals with the development of this lymphoid organ as observed by light and electron microscopy. The anal tonsil was characterized by the initial postnatal development. On neonatal Day 1, a pair of epithelial crypts formed at the dorsal boundary between the anus and the ostium urogenitoanale. On Day 2 after birth, lymphocytes began to accumulate in the subepithelial mesenchymal tissue under the crypt. From Day 3 on, the lymphocytes increased to form a lymph nodule, from which, on Day 5, some lymphocytes began to penetrate into the crypt epithelium. The crypt and the nodule were fused together between Days 6 and 8. A germinal center-like structure was observed on Day 20 after birth. Around Day 40, the invading cells comprised cellular units consisting of large and small lymphocytes and plasma cells. High endothelial venules were observed in the parafollicular area at this time. These findings indicate that the anal tonsil originates from an accumulation of lymphocytes in the mesenchymal tissue close to a particular epithelium of the crypt, presumably in response to antigens in foods; the tonsillar structure is then gradually completed by fusion of the lymphoid and epithelial elements. This paper further reports on an electron microscope finding on Day 8 where the anal tonsillar crypt epithelium was seen to contain some basal-granulated cells of the open type.

In the laboratory shrew, the rectum, urethra and vagina do not open independently, but share their openings into a large common cavity, the ostium urogenitoanale. The surface of the ostium urogenitoanale is covered by a stratified flattened epithelium,

and its narrowed orifice opens to excrete feces and urine or to copulate (Fig. 1). We identified a new tonsil-like structure associated with the ostium urogenitoanale in the laboratory shrew, i. e., at the distal end of the gut (KUBO and ISOMURA, 1996). On the exactly opposite, oral end of the gut of this animal, tonsillar structures are known to occur near the fauces; their postnatal development, including their functional roles, has been described previously (TAKAGI et al., 1985; KIMURA et al., 1989, 1992, 1996; TOHYA and KIMURA, 1992).

The present study deals with the hitherto unknown development of anal tonsils in the laboratory shrew. We made a light and electron microscopic observation on the postnatal development of the anal tonsils, as our preliminary study had indicated that the tissue in question first appears after birth.

MATERIALS AND METHODS

Thirty-five laboratory shrews (*Suncus murinus*) of either sex were used on Days 1, 2, 3, 5, to 11, 15, 20, 40, and 50 after birth. They were bred in our laboratory animal center in an air-conditioned room (room temperature: $24 \pm 1^\circ\text{C}$, humidity: $55 \pm 5\%$). Under anesthesia with an intraperitoneal injection of sodium pentobarbital (60 mg/kg), the animals were first perfused with 5 ml of 0.1 M phosphate-buffered saline (pH 7.4) through the left cardiac ventricle, and then with an ice-chilled fixative containing 4% paraformaldehyde and 1% glutaraldehyde buffered with 0.1 M phosphate (pH 7.4). After sufficient fixation, the caudal half of each animal was removed with scissors. All the tissues around the ostium urogenitoanale

*This study was supported by a Grant-in-Aid from Fujita Health University, Japan.

Fig. 1. Schematic representation of the anal (AT) and vaginal tonsils (VT) associated to the ostium urogenitoanale (O), and anus (A) and vagina (V) in the laboratory shrew. **a.** Ventral view. Muscles and skin of the abdominal part were removed. **b.** Horizontal figure at the level of the anal tonsil. **c.** Midsagittal figure of the ostium urogenitoanale. B urinary bladder, R rectum, U urethra, UT uterus

—except the skeletal elements of the pelvis— were then carefully removed with scissors and forceps under a dissecting microscope and immersed in the same fixative for 24 h. They were rinsed in a 0.1 M phosphate buffer containing 10, 20, and 30% sucrose for 3 days. After this, 10 μ m serial sections were made in a cryostat and divided into two groups for light (LM) and transmission electron microscopy (TEM), to compare adjacent sections by different methods. The sections subjected to hematoxylin-eosin staining for LM were examined for the location of the tonsils. After confirming the location of the tonsils, the sections in which an anal tonsil was included were selected to be fixed in 2% osmic acid for 30 min. They were then dehydrated through graded ethanol concentrations, and embedded in Epon 812. Ultrathin sections were made with an ultramicrotome, stained with uranyl acetate and lead citrate, and examined under a JEM 2000FX electron microscope (JEOL, Tokyo).

The volume of the anal tonsil was measured on serial sections stained with hematoxylin and eosin using an image analyzer (Nikon LUZEX FX) connected to a personal computer.

RESULTS

On Day 1 after birth, a pair of deep dorsal crypts consisting of three or four layers of epithelial cells was formed at the dorsal boundary between the anus and the ostium urogenitoanale; few lymphoid cells were recognized around the crypt (Fig. 2a, b arrow).

On Day 2, several dozens of lymphocytes were seen to be gathered in the connective tissue surrounding the crypt (Fig. 2 c, d arrow).

On Days 3–5 after birth, numbers of lymphoid cells were accumulated in the subepithelial layer under the basement membrane of the crypt, and began to form a lymphonodule. On Day 5, the volume of the nodule measured approximately 0.002 mm³ (Fig. 2 e arrows, f). TEM indicated that several large lymphocytes

Fig. 2. Three different stages in the development of anal tonsil as demonstrated in a transverse section of the ostium urogenitoanale (*O*) stained with hematoxylin-eosin. **a.** Postnatal Day 1. Overview of the epithelium of the ostium extending a crypt at its righthand end. **b.** Closer view of Figure 1a. Few lymphoid cells are seen around the crypt at this stage (*arrow*). **c.** Postnatal Day 2. The configuration of the ostium is now more complicated and the crypts are evident on both sides. **d.** Closer view of Figure 1c. Numerous lymphoid cells are observed around the crypt of the ostium urogenitoanale at this stage (*arrow*). **e.** Postnatal Day 5. A large accumulation of lymphoid cells is seen near the end of the crypt (*arrow*). **f.** Closer view of Figure 1e. Lymphoid cells are gathered to form an anal tonsil (*A*). No structure suggesting a germinal center is recognized at this stage. *a, c, e:* $\times 25$; *b, d, f:* $\times 150$

Fig. 3. Electron micrographs showing the penetration of lymphocytes into the tonsillar crypt epithelium (E) on Day 3. B arrowhead basement membrane, SE subepithelial area. **a.** Large lymphocytes (arrows) in the epithelium. $\times 3,400$. **b.** A small lymphocyte (arrow) into the epithelium apparently associated with another lymphoid cell. $\times 3,900$

Fig. 4. The anal tonsil (*A*) on Day 7 as shown in a transverse section of the ostium urogenitoanale (*O*). *C* tonsillar crypt. **a.** Overview. A well-developed lymph follicle is associated with the crypt on either side (*arrows*). $\times 25$. **b.** Closer view of a lefthand portion of Figure 3a. The accumulation of the lymphocytes is now fused with the crypt epithelium. Several capillaries (*arrows*) are seen in the lymphoid tissue. $\times 150$. **c.** Higher magnification of the epithelial layer in Figure 3a. Some small invading cells (*arrows*) are observed in the epithelium. $\times 520$

Fig. 5. Legend on the opposite page

Fig. 6. Light micrographs of anal tonsils at more advanced stages of development as shown in a transverse section of the ostium urogenitoanale (O). C tonsillar crypt. **a.** Postnatal Day 50. A large anal tonsil (arrows) is seen surrounding the tonsillar crypt on either side. $\times 25$. **b.** Postnatal Day 40. Note light germinal center-like structures (*). $\times 75$. **c.** Higher magnification of a portion of the cryptal epithelium shown in Figure 5b. In the epithelium many large invading cells (arrows) are observed. $\times 800$

Fig. 5. Electron micrographs of a basal-granulated cell found in the tonsillar crypt of an animal on Day 8. **a.** Overview of the light pyramidal cell shows granules in the basal cytoplasm (arrows), and its extending to the crypt space. M microvillous tuft. $\times 3,800$. **b.** Closer view of the supranuclear area of the cell shown in Figure 4a. Rich polysomes, numerous mitochondria and a pair of centrioles (C) are noted. The cell and the neighboring epithelial cell are joined by desmosomes (arrowheads) $\times 10,000$. **c.** Basal portion of the cell shown in Figure 4a. The granules are dense-cored in type and bounded with a limiting membrane (arrows). Desmosomes are again observed (arrowheads). $\times 10,000$

Fig. 7. Legend on the opposite page.

(Fig. 3 a arrows) and small lymphocytes (Fig. 3 b arrow) began to penetrate into the epithelial layer through the basement membrane at this time.

Between Days 6 and 8, the lymph nodule and epithelial layer fused together (Fig. 4 a arrows, b), and several blood capillaries were recognized in the marginal zone of the nodule (Fig. 4 b arrows). The crypt now was composed of about 7–8 layers of epithelial cells, which were densely surrounded by numerous lymphoid cells (Fig. 4 a, b, c). Clear cells of smaller and larger sizes were observed invading the epithelium (Fig. 4 c arrows). TEM observation confirmed that the invading cells were small and large lymphocytes mixed with a few neutrophils.

On Day 6 after birth, the crypt epithelium contained a few large pale pyramidal cells including many secretory granules, especially cored-granules in their basal cytoplasm (Fig. 5 a, c). The tapered apex of the cell was equipped with a microvillous tuft which was housed in a groove of the superficial flattened epithelial cells. The cells were connected to neighboring epithelial cells by desmosomes (Fig. 5 b, c).

The volume of the anal tonsil on Days 15–20 after birth was about 0.4 mm³, which was approximately half the size in the adult. The volume on Day 50 ranged between 0.8 and 1.0 mm³, and that corresponded to the volume in the adult.

On Days 40–50, the anal tonsil comprised a deeper crypt and one or two lymph nodules, and had attained adult structure and size (Fig. 6 a, b). The lymphocytes were gathered more densely in the cortical region of the tonsil, and the central region—which appeared lighter—seemed to represent a germinal center (Fig. 6 b*). However, as this germinal center was not clear as compared with that of the human faucial tonsil and lymph node, we designated it a germinal center-like structure. A number of larger and smaller cells were observed invading the epithelium (Fig. 6 c). TEM observation showed that the invading cells formed a cellular unit consisting of plasma cells, and large and small lymphocytes (Fig. 7 a, b). The cytoplasm of the plasma cells was filled with well-developed rough endoplasmic reticulum and mitochondria (Fig. 7 b), and sometimes included a Russell's body. Large lymphocytes invading the epithelium were elongated in shape.

The germinal center-like structure in the lymphatic follicle consisted of large and medium lymphocytes

and reticular cells (Fig. 8 b), and was distinguishable from the parafollicular areas in the lymphatic follicles (Fig. 7 a). Many small lymphocytes and reticular cells were observed in the parafollicular area, where high endothelial venules (HEVs) were also recognized (Fig. 8 c). The wall of the HEVs was not so high as that in human tonsillar tissue (Fig. 8 c). Lymphocytes were observed to pass both individually (Fig. 8 c arrow) and collectively (Fig. 8 c) through the endothelial cell wall. Some of the lymphocytes in the lumen of the HEV were in contact with its endothelial cells (Fig. 8 c arrowheads).

DISCUSSION

We confirmed our previous finding concerning the occurrence of a pair of tonsils at the boundary between the anus and the ostium urogenitolanale in the laboratory shrew, *Suncus murinus* (KUBO and ISOMURA, 1996), and now demonstrated their development.

Many studies have reported on the development of the faucial tonsils in various species. In humans, at fetal stages after the 14th gestational week, the mesenchyme underlying the tonsil cavity is invaded by mononuclear wandering cells (GAUDECKER and MÜLLER-HERMELINK, 1982). In the rabbit faucial tonsil, the motile lymphoid cells have been observed to occur in the tonsillar area observed on Day 22 of gestation (LEENE, 1971). In contrast, in the faucial tonsils of the laboratory shrew, it has been shown that a specific kind of large lymphoid cells moved first on Day 3 after birth through the basement membrane into the epithelium, and between Days 5 and 7, the lymphocytes formed clusters under the epithelium (TOHYA and KIMURA, 1992). In summary, while human and rabbit tonsils develop during gestation, the faucial and anal tonsils of the house shrew begin to form only after birth. Reasons for this difference in the tonsil differentiation between the human or rabbit and the house shrew are not clear. It seems interesting to note that antigen-stimulation by intake of food after birth seem to be necessary in the laboratory shrew for the formation of the tonsils, whereas for the faucial tonsils in humans and rabbits, these organs apparently are prepared prior to their encounter with antigens in food.

Our findings indicate that the anal tonsil originates

Fig. 7. Electron micrographs of the tonsillar crypt epithelium, postnatal Day 50. **a.** The crypt epithelium contains cell colonies consisting of some large (L) and small lymphocytes (S) and an immature plasma cell or immunoblast (IB). $\times 2,400$. **b.** A lymphoid cell colony consisting of plasma cell (P), an immunoblast (IB) and some lymphocytes. $\times 2,600$

Fig. 8. Electron micrographs showing different areas of an anal tonsil at Day 50. **a.** A parafollicular area of the lymphatic nodule containing tightly gathered, small lymphocytes (S). $\times 2,500$. **b.** A germinal center-like area showing large lymphocytes (L) and reticular cells (R). $\times 2,500$. **c.** A high endothelial venule in the parafollicular area. The lymphocytes are passing through the capillary endothelium (arrow). S small lymphocytes covered by a sheet of endothelial cytoplasm, arrowheads small lymphocytes in contact with the endothelium. $\times 2,500$

from an accumulation of lymphocytes in a mesenchymal lymphatic tissue, which is believed to belong to the category of gut-associated lymphoid tissue (GALT), together with the lymphatic nodules, Peyer's patches and the appendix (FICHELIUS, 1969; OWEN and JONES, 1974). The structure of the anal tonsil differs from that of the central lymphoid tissue. In the house shrew, which has no appendix and does not develop Peyer's patches (HANAMURA et al., 1985), the anal tonsil is presumed to function in place of those gut-associated lymphoid organs. The present findings on the movement of the tonsil tissue to the crypt epithelium associated with the immigration of lymphoid cells, consisting of plasma cells and lymphocytes, closely correlate with previously known features in the faucial tonsil (HOWIE, 1980; NAIR and ROSSINSKY, 1984; OLÁH et al., 1988; PERRY, 1994; BELZ and HEATH, 1996; BELZ, 1998).

The present study demonstrated that the anal tonsil crypt of the laboratory shrew contained clear cells with basally located secretory-like granules. The cells extended their process between the superficial cells of the epithelium, and its apical end apparently was microvillous in structure. From these cytological features, the cells were identified with basal-granulated cells of the open type (KOBAYASHI et al., 1970; FUJITA and KOBAYASHI, 1973) which are known to occur in extensive areas of the digestive tract in different species including human colon and rectum (OSAKA et al., 1971), and to release peptide hormones and amines from their cell base, and to regulate the motility and secretion of the intestine. This is the first report that the basal-granulated cells occur in the anal region and in the laboratory shrew. The basal-granule cells are known to comprise various cell types producing different gastro-intestinal hormones (review: FUJITA and KOBAYASHI, 1977). Further studies are needed to examine the type and function of the basal-granulated cells observed in the anal tonsils.

REFERENCES

- BELZ, G. T.: An unusual structure of venules in tonsils of the soft palate of young pigs. *J. Anat.* 192: 131-135 (1998).
- BELZ, G. T. and T. J. HEATH: Tonsils of the soft palate of young pigs: Crypt structure and lymphoepithelium. *Anat. Rec.* 245: 102-113 (1996).
- FICHELIUS, K. E.: The lymphoid organs—a new tool at the lymphoepithelial organs. In: (ed. by) L. FIOREDANATI and M. G. HANNA: Lymphatic tissue and germinal centers. Prentice Hall, New York, 1969 (p. 9-20).
- FUJITA, T. and S. KOBAYASHI: The cells and hormones of the GEP endocrine system—The current of studies. In: (ed. by) T. FUJITA: Gastro-entero-pancreatic endocrine system. A cell-biological approach. Igaku Shoin, Tokyo, 1973 (p. 1-16).
- FUJITA, T. and S. KOBAYASHI: Structure and function of gut endocrine cells. *Int. Rev. Cytol. Suppl.* 6: 187-233 (1977).
- GAUDECKER, B. and H. K. MÜLLER-HERMELINK: The development of the human tonsilla palatina. *Cell Tiss. Res.* 224: 579-600 (1982).
- HANAMURA, H., H. MINEDA, M. KUROMARU, J. KITOH, T. MIYAKI, G. ISOMURA and K. YAMASHITA: The digestive organs (In Japanese). In: (ed. by) K. KONDOH, S. ODA, J. KITOH, K. OHTA and G. ISOMURA: *Suncus murinus*. Gakkai Shuppan Center, Tokyo, 1985 (p. 210-228).
- HOWIE, A. J.: Scanning and transmission electron microscopy on the epithelium of human palatine tonsils. *J. Pathol.* 130: 92-98 (1980).
- KIMURA, M., K. TOHYA and K. KUKI: Laboratory suncus: A new model animal for research. *Acta Otolaryngol. Suppl.* 523: 20-24 (1996).
- KIMURA, M., K. TOHYA and T. TABATA: Scanning, transmission and immunoelectron microscopical studies of tonsil-like lymphoid organ of normal and horseradish-peroxidase-injected laboratory suncuses. *Acta Anat.* 136: 177-184 (1989).
- KIMURA, M., K. TOHYA and T. TABATA: Phylogenetic and ultrastructural properties of the primitive tonsils of laboratory suncuses, Tonsils: A clinically oriented update. *Adv. Otorhinolaryngol.* 47: 21-27 (1992).
- KOBAYASHI, S., T. FUJITA and T. SASAGAWA: The endocrine cells of human duodenal mucosa. An electron microscopic study. *Arch. Histol. Jap.* 31: 477-494 (1970).
- KUBO, K. and G. ISOMURA: Presence and structure of anal and vaginal tonsils in the laboratory shrew (*Suncus murinus*). *Ann. Anat.* 178: 251-258 (1996).
- LEENE, W.: Origin and fate of lymphoid cells in the developing palatine tonsil of the rabbit. A possible mechanism of homing of lymphoid cells. *Z. Zellforsch.* 116: 502-522 (1971).
- NAIR, N. and K. ROSSINSKY: Crypt architecture of tonsilla lingualis in the monkey, *Macaca fascicularis*. *Cell Tiss. Res.* 237: 619-627 (1984).
- OLÁH, I. L., L. TAKÁCS and I. TÖRÖ: Formation of lymphoepithelial tissue in the sheep's palatine tonsil. *Acta Otolaryngol. Suppl.* 454: 7-17 (1988).
- OSAKA, M., T. SASAGAWA, S. KOBAYASHI and T. FUJITA: The endocrine cells in the human colon and rectum. An electron microscope study of biopsy materials. *Arch. Histol. Jap.* 33: 247-260 (1971).
- OWEN, R. and A. JONES: Epithelial cell specialization within human Peyer's patches: an ultrastructural study of intestinal lymphoid follicles. *Gastroenterology* 66: 189-203 (1974).
- PERRY, M. E.: The specialized structure of crypt epithelium in the human palatine tonsil and its functional significance. *J. Anat.* 185: 111-127 (1994).

- TAKAGI, T., K. NISHIKAWA, H. IGARASHI and K. NOBUKI:** Problems on the definition of a palatine tonsil with special reference to the suncus tonsil. *Jap. J. Tonsil.* 24: 202-207 (1985).
- TOHYA, K. and M. KIMURA:** Ultrastructural study of post-natal development of the tonsillar crypt epithelium of the musk shrew, *Suncus murinus*. *Anat. Embryol.* 186: 335-340 (1992).

Dr. Kazuyoshi SAKAI
Department of Anatomy
Fujita Health University, College
1-98 Dengakugakubo,
Kutsukake-cho, Toyoake
470-1192 Japan
Tel: +81-562-93-9434
Fax: +81-562-93-0290
E-mail: ksakai@fujita-hu.ac.jp

酒 井 一 由
470-1192
愛知県豊明市杣掛町田楽ヶ窪 1-98
藤田保健衛生大学短期大学
人体構造学