Incorporation of instilled *Candida albicans* and a lipopolysaccharide into the palatine tonsil of rabbit

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Abstract: The palatine tonsil is thought to be the organ which accepts antigens to initiate an immunological response, but the incorporation of the antigens from the oral cavity was not yet known. To show this incorporation, fluorescence-labeled *Candida albicans* and a lipopolysaccharide were instilled around the rabbit palatine tonsil. The distribution of fluorescence was examined in frozen sections of the tonsil after 30, 60 and 180 minutes of the instillation. *Candida albicans* and a lipopolysaccharide were incorporated into the cryptoepithelial tissue of the palatine tonsil within 30 minutes. The antigens in the epithelium were transported to the intratonsillar follicles and partly to the deep cervical lymph nodes after 60 minutes. The lipopolysaccharide was found intrafollicular by both in the tonsil and the deep cervical lymph nodes earlier than *Candida albicans* was. Because the normal cryptoepithelium easily passed through the external antigens which were transported to the neighboring lymphoid follicles, it was suggested that the cryptoepithelium was the entrance of the immunological response in the palatine tonsil and neighboring lymphoid tissues in the normal condition.

Introduction

The palatine tonsil is one of the infra-epithelial lymphatic tissues which directly contacts external antigens through the epithelium. The infra-epithelial lymphatic tissues include the appendix and the Peyer’s patches, which are called gut-associated lymphoid tissues (GALT). It was believed that GALT incorporated antigens and the lymphocytes in GALT were transported to the intestinal mucosa and produced antibodies which were secreted from the mucosa and from remote exocrine glands. Similar to GALT, the tonsil may incorporate antigens to initiate an immunological response. Although it was speculated some information on
antigens in the intra-oral cavity was caught in the palatine tonsil and then transported to the other systemic lymph tissues through the lymph vessels, the details of this were not yet clear\(^5\). So far, many reports have showed that in the inflamed tonsil, soluble antigens pass through the epithelium, which is reticulated because of inflammation, in a short time\(^6\)–\(^9\). For the granular antigens, such as pyogenic bacteria, one day or more was required to invade the tonsillar tissue\(^14\)–\(^18\). One day may be enough to cause the inflammation in the epithelial tissue which became reticulated. Thus, we do not know whether antigens in the oral cavity may access freely the normal tonsillar tissue and whether there are any differences in the distribution of antigens differing in size.

In the present experiment, we chose Candida albicans (C. albicans) as one of the biggest antigens among the microbes existing in the oral cavity, and a lipopolysaccharide (LPS) as a bacterial small component. These were instilled around the palatine tonsil, and we examined the distribution in the tissue section at the appropriate times. We found that C. albicans and LPS passed through the tissue very easily and some information of the antigens may be presenting in the tonsil and in neighboring lymph nodes.

**Materials and methods**

1. **Rabbits**

The animals used in the experiments were 8 weeks old male Japanese albino rabbits, weighing from 1.0 to 1.2 kg.

2. **Preparation of antigens**

C. albicans ATCC 18804 successively preserved in our laboratory were cultured in brain heart infusion broth (Difco Lab.) at 37°C for 18 hours, washed two times with sterilized saline solution and centrifuged (1,200 × g, 20 min). The cells were labeled with PKH 26 (fluorescent staining kit, Zynaxis)\(^19\) and suspended in sterilized saline (1 × 10⁷ cells/ml).

Fluorescein isothiocyanate (FITC) labeled LPS
from *Escherichia coli* (serotype 0111: B4) (Sigma) was added with sterilized saline to adjust to 10 μg/ml, and was sterilized by filtration.

3. Antigens application
FITC-labeled LPS and PKH 26-labeled *C. albicans* (0.5 ml each) were instilled around the rabbit palatine tonsil under intravenous anesthesia with sodium pentobarbital. At 30, 60 and 180 minutes after the instillation, each rabbit was killed painlessly by excessive intravenous administration of sodium pentobarbital. The palatine tonsil and the deep cervical lymph nodes were excised, embedded in CRYO-M-BED (BRIGHT Instrument) and rapidly frozen. The frozen tissues were sectioned at 8 and 30 μm in thickness.

4. Observation with fluorescence
The frozen sections were fixed with 2.5% glutaraldehyde for 5 minutes, washed two times with distilled water, and air-dried at room temperature in the dark. The sections were mounted with 90% glycerol in phosphate buffered saline (PBS) containing 1% *p*-phenylendiamine. The distribution of fluorescence-labeled antigens was examined with a fluorescent microscopic apparatus (New Vanox AH2-FL, Olympus) and a confocal type of scanning laser-biological microscopy system (LSM-GB 200, Olympus).

Results

1. *Candida albicans* application
Thirty minutes after the application of *C. albicans*, labeled yeasts were observed at the lymph-epithelial symbiotic part in contact with the cryptocavity and in the deep area of the symbiotic part of the palatine tonsil (Fig. 1-A). The result showed that the *C. albicans* passed through the epithelial tissue within 30 minutes. Many labeled *C. albicans* were engulfed by the cells at the same site (Fig. 1-B). It was suggested that many phagocytes as macrophages were located in the epithelial tissue and phagocytosed the microorganisms in the normal condition.

Sixty minutes after the application of *C. albicans*, the distribution of the yeasts in a part of the lymph-epithelial symbiotic part and the infraepitheliocortical layer was similar to that of 30 minutes after the application of *C. albicans*. Labeled yeasts were also found in the part of the cap area at the epithelial side surrounding the follicles (Fig. 2).

One hundred and eighty minutes after the application of *C. albicans*, a few yeasts of free *C. albicans* was detected in the palatine tonsil, and most labeled yeasts were detected in the cells widely distributed in the deep cortical layer (Fig. 3-A). Some of the cells engulfing the *C. albicans* were localized in the part of cortex laying around the follicles, and were seen in the area of the germinal center (Fig. 3-B). Furthermore, the cells engulfing the *C. albicans* were seen in the afferent lymphatics around the deep cervical lymph nodes (Fig. 3-C).
2. LPS application

30 minutes after LPS application, most of the fluorescence was found in the cells which were widely distributed in the cortical layer including the lymphoid follicles of the palatine tonsil. Some amorphous fluorescence was seen in part of the area between the connective tissue surrounding the capsule and the efferent lymphatics (Fig. 4).

60 minutes after the application of LPS, the distribution of LPS in the palatine tonsil was similar to that after 30 minutes of the application of LPS. In the deep cervical lymph nodes, the cells containing fluorescence were found in the afferent lymphatics and marginal sinus (Fig. 5).

180 minutes after the application of LPS, the cells containing fluorescence were found in and out of the lymphoid follicles, and in the germinal center of the palatine tonsil. Fluorescence in the connective tissue layer was greater than that after 30 and 60 minutes of the application of LPS (Fig. 6-A). In the deep cervical lymph nodes, the cells including fluorescence were widely distributed in the cortical layer including the lymphoid follicles (Fig. 6-B).

Discussion

We showed here first that soluble antigens were incorporated into the normal tonsillar tissue. In the LPS application, the fluorescence was incorporated into the cells which moved in the tonsil in a similar fashion to that in the C. albicans application.

Although several kinds of proteins incorporated into the palatine tonsil, these reports were based on the data of the tonsil after sensitization in rabbits or on the data of human with inflammation of the tonsil. In the cases of habitual tonsillitis, it was
Phagocytes which engulfed LPS and free LPS were widely distributed in the cortical layer containing the lymphoid follicles, and LPS in the intercellular space in the connective tissue around the capsule. Bar = 50 μm. ○ = fluorescence shown that antigens easily passed through the cryptoeptithelium because of the progress of the reticular formation of the epithelium\(^1^2\).

We showed that \textit{C. albicans} incorporated into the tonsil within 30 minutes. After the instillation of \textit{Staphylococcus aureus} at the same site 2 or 3 times a day\(^1^4\) or after the topical application of india ink by brush to the palatine tonsil surface\(^2^1\), the granular antigens passed through the epithelial tissue in one day and reached to a wide area between the infrapithelium and the capsule. Although Hatano et al. and Haraguchi et al. did not report the incorporation within one day, the bacterial cells possibly did within 30 minutes. Because the diameter of \textit{C. albicans} was several times greater than that of \textit{Staphylococcus aureus} and india ink, the incorporation of the bacteria seems to be easier than that of the yeasts. Applying \textit{Streptococcus pyogenes} by the spraying around the palatine tonsil, the bacteria adhered, but did not invade the cryptoeptithelium\(^1^5\). It seemed that the incorporation ratio of bacterial cells to the tonsil depended on cell density of bacterial cells at the crypt of the tonsil, rather than on the species specificity. Application by instillation or topical application by brush will provide a higher cell density in small area than that by spray. The present result shows that the cryptoeptithelium has a structure passing through a large antigen nearly 3~5 μm in diameter like the yeast form of \textit{C. albicans}.

At the lymph-epithelial symbiotic part, a number of microcrypts were founds and were classified into
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The tonsil contains three types of microcrypts named MC-3, MC-1, and MC-2. MC-3 has a similar structure to M-cells which incorporate antigens into intestinal mucosa. MC-1 and MC-2 have small pores. It is likely that antigens instilled around the palatine tonsil passed through the cryptoeplithelium from one of these microcrypts to the tonsillar parenchyma.

We showed that the site of fluorescence shifted from the epithelial side to the cortical area, the surroundings of lymphoid follicles, and the germinal center with the time after application of C. albicans. It was suggested that phagocytes moved to these tissues after phagocytosing the yeasts. Since the major phagocytes which appeared in the tonsillar epithelium and the cortical layer were macrophages, some macrophages must engulf bacterial cells.

There are two transferring pathways of antigens from the palatine tonsil to the deep cervical lymph nodes. The first pathway is through lymphatics and the second one is through blood vessels. It was shown that some pigments which were injected into the lymph vessels of the human palatine tonsil moved to the middle-deep cervical lymph nodes through the efferent lymphatics surrounding the capsule. On the other hand, it was reported that horseradish peroxidase reached the deep cervical lymph nodes by the stream of blood circulating through the high endothelial venules. In the present study, phagocytes were first seen in lymph sinuses distributed in the interfollicular area of the tonsil and then in the afferent lymphatics of the deep cervical lymph nodes in both C. albicans and LPS application. The result showed that the antigens incorporating into the tonsil were transferred through the efferent lymphatics to the deep cervical lymph nodes by phagocytes.

In sensitized tonsils, antibody producing cells and specific antibodies were detected in the mixed tonsillar cell culture with splenic cells and in the tonsillar cell culture. In the sensitized tonsils, by topical application and intra-cryptocavitary infusion, specific antibodies were produced in all cases.

We supposed that some antigen information should
be presented to T cells which helped B cells to produce the antibodies. This proposal was made from the following observations. First, the incorporated antigens were phagocytosed efficiently. We suggest the major phagocytes were macrophages which were one of the antigen presenting cells. Second, phagocytes moved around the follicles, especially localized in the part of the cap area at the epithelial side of the follicles. It is believed that antigen presenting cells transmit the antigen information at the cap area where the dominant cells are T cells, which are believed to be special cells which accept information from antigen presenting cells. Third, a few antigen bearing cells moved to the germinal center, where B cells proliferate and become antibody producing cells.

We did not show the transfer of the information and the intensity of the stimuli to initiate the immunological response from the present study. The results show that many phagocytes were located at the area surrounding the follicles, and supports the idea that a single instillation is enough to sensitize the tonsillar cells to become antibody producing cells.

References

19) Horan, P.K., Melnicoff, M.J., Jensen, B.D. and