NOTE Internal Medicine

Attachment of Malassezia pachydermatis to the Ear Dermal Cells in Canine Otitis Externa

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ABSTRACT. To investigate the predominance of Malassezia pachydermatis (M. pachydermatis) as a causative agent of canine otitis externa, ear cerumen samples were observed for adhesion of M. pachydermatis to the cornified epithelial cells by light and electron microscopes. The yeasts appeared not to adhere to the cornified epithelial cells directly, but they seemed to exist in the proximity of the epithelial cells with an electron opaque halo-like space around them. Polysaccharide and lipid staining techniques were conducted to identify the substances existing in that space. Lipid substances, not saccharides, were observed around the yeasts and the cornified epithelial cells. These results suggested that in the canine ear canal malassezia yeast attachment to the cornified epithelial cells is mediated by lipids.

KEY WORDS: canine, Malassezia pachydermatis, otitis externa.


In Japan, the relative incidence of canine otitis externa was 4.4% of all canine diseases in 1993, but in 1997 it had increased to 8.1%, its highest recorded incidence rat [5]. Malassezia pachydermatis (M. pachydermatis) has been isolated from the cerumen samples of otitis externa-affected dogs and is involved as a causative agent [2, 8]. M. pachydermatis is the most common organism found in ear specimens even in healthy dogs, accordingly this organism has been recognized as an opportunistic pathogen [3]. However, it remains to be investigated why M. pachydermatis selectively inhabits the ear canal of dogs and under what conditions it grows to cause otitis externa. Almost all microorganisms present host and site specificity and these depend upon microenvironmental factors such as host cell adhesion, nutrients for growth, growth inhibitors, pH and oxidation reduction potential [1]. M. pachydermatis yeasts associated closely with epithelial cells are commonly seen under the microscope in observation of cerumen smears from otitis externa (Fig. 1). If specific adherence of M. pachydermatis to epithelial cells is certified in survival and growth in the ear canal, new methods of treatment and prevention may be derived. Up to the present, however, published data showing evidence for yeast adherence to the cornified epithelial cells is not available. We therefore investigated the attachment of M. pachydermatis to the dermal epithelial cells of the ear canal by light and electron microscope observations of the ear cerumen specimens.

In order to study the association between the yeasts and the cornified epithelial cells, specimens for microscopy were taken from otitis externa cases with M. pachydermatis infections. Pieces of cerumen were fixed with paraformaldehyde and glutaraldehyde for 90 min at 4°C, then treated with 2% osmium tetroxide for 90 min at 0°C. Separately a treatment with potassium permanganate was used to identify the participation of saccharides in the possible adherence of M. pachydermatis to the cornified epithelial cells. After dehydration with ethyl alcohol the specimens were embedded in L. R. White resin (London Resin Co., Ltd., U. K.). For the light microscope examinations, thin sections (0.5–1 µm in thickness) stained with 1% toluidine blue and PAS stain were prepared. Ultra-thin sections (0.05–0.1 µm in thickness) of the same specimens were stained with uranyl acetate and lead citrate, and examined with a transmission electron microscope (Hitachi Co., Ltd., Tokyo, Japan) at 75 kv to determine the features between the yeasts and the epithelial cells.

If M. pachydermatis can adhere or attach to the host cell surface, the evidence might appear on microscopic examination. Light microscope pictures of toluidine blue staining (Fig. 1) showed that the yeasts attached themselves to the cornified epithelial cells of the ear canal.

Fig. 1. In a cerumen smear from canine otitis externa, malassezia yeasts appear to be associated with epithelial cells (Giemsa stain, × 1,000).
epithelial cells, and seemed to be surrounded by capsules. Electron microscope pictures were expected to show the mechanism of attachment, but they did not indicate any structure such as glycocalyx accounting for the attachment. One of the electron microscope pictures (Fig. 3) revealed capsule-like low density areas surrounding the yeasts in the promixity of the cornified epithelial cells. PAS and Alucian blue staining (photographs not presented) showed only the saccharide wall of the yeasts. The substances in the spaces between the yeasts and the cornified cells were not stained by these staining methods.

Since lipids can be solved away during the staining techniques, the electron opaque halo-like space was considered to have been filled with lipids. To avoid the lipid elution and to examine the substances between the yeasts and the cornified epithelial cells, other clinical cerumen samples were fixed with formalin and frozen sections (6 µm in thickness) were made by a cryostatic microtome. These sections were stained with Alucian blue for polysaccharides and Oil red O for lipids. One of the microscope pictures (Fig. 4) of an Oil red O stained section looks like the yeast cells were embedded in lipid substances; Alucian blue stained samples showed only the blue wall of yeasts (photographs not presented).

It is essential that organisms adhere to host cells before their growth and their pathogenicity can become manifested. For instance, since the intestinal contents always flow down, bacteria have to adhere to the intestinal surface for growth. Not only in the intestine but also in the ear canal, microorganisms must adhere to the surface, because even ear exudates are always moving toward the exterior [4]. The appearance of *M. pachydermatis* in association with cornified cells as in Fig. 1 is observed in smear samples of canine otitis externa, and at the same time some yeasts are found separately. Ordinal phenomena like this suggest possible adhesion of the yeasts to the cornified cells.

Curious findings concerning the association between malassezia yeasts and the cornified epithelial cells in the cerumen sections were observed. The microscopic images showed capsule-like spaces surrounding the yeasts and this space proved to be filled with a lipid substance. The cornified epithelial cells had many subsidences and the capsuled yeasts looked to be buried in the subsidences.

*M. pachydermatis* in the canine ear canal has been assumed to develop from opportunistic infections when its microenvironment is changed [6]. This organism is commonly found even in the healthy ear. The significant correlation between the populations of yeasts and the cornified cells was regarded as a certain association of the yeasts to the host cells as in bacterial adhesion to the host cells. However, in the ultrastructural investigations, the adhesion between the organisms and the host cornified cells was show to be an unlikely type of bacterial adhesion. The cerumen sections stained with Oil red O showed abundant lipids on the surface of the cornified cells and around the yeasts, and lipids secreted by the apocrine glands in the ear canal might play a role in making the yeasts attach to the host epi-

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**Fig. 2.** A cerumen sample from otitis externa with *M. pachydermatis* in a dog, stained with toluidine blue (× 1,000). Some yeasts appear to be held by cornified cells.

**Fig. 3.** Ultra-thin sections (0.05–0.1 µm in thickness) were made from a cerumen sample from a case with *M. pachydermatis*, and observed by an electron microscope. The picture (× 30,000) shows a low-density space between the yeasts and the cornified cells, but no mechanism of adhesion was revealed in the image.

**Fig. 4.** Frozen sections (6 µm in thickness) were made from a formalin-fixed cerumen sample from a case with *M. pachydermatis* otitis externa, and stained with oil-red O and hematoxylin eosin. Yeasts (arrows) are enveloped by lipids between layers of cornified epithelial cells (Ep) (× 1,000).
thelial cells. After the attachment of the yeasts, the organisms will require special factors for growth and the clinical appearance of otitis externa, lipids in the ear wax have been suggested as enhancing the growth of *M. pachydermatis* [7].

The present morphological observations indicated that malassezia yeasts float in lipids, are carried among the layers of cornified cell surfaces, and proliferate in the lipids. Finally, the cornified cells formed subsidences by their budding. The lipid layer must have an important role by its stickiness and nutriments in supporting attachment and proliferation of malassezia yeasts. This mechanism should be examined for the development of future methods for controlling malassezia-infected otitis externa in dogs.

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REFERENCES