Significance of Langerhans' Cells in Middle Ear Cholesteatoma

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Recent advances in immunology have opened a new approach to investigating the etiology and pathogenesis of aural cholesteatoma by the immunohistochemical technique. Immunohistochemical and submicroscopic analysis of human cholesteatoma matrices revealed the presence of Langerhans' cells. Several reports have suggested that Langerhans' cells in cholesteatoma are significant, and that the pathogenesis of this disease including bone resorption could be explained as a cell-mediated immune response, but this is still controversial. In this study, Langerhans' cells in cholesteatoma were quantitated and compared with those in postauricular skin and in skin of the open mastoidectomized cavity. The results did not support the hypothesis that Langerhans' cells have a primary role in the development of aural cholesteatoma.

Key Words: Cholesteatoma, immunohistochemistry, Langerhans' cells

Otolologists have been engaged in a search for answers concerning the pathogenesis and pathophysiology of cholesteatoma since its early descriptions by Cruveilheir (1829), and Mueller (1838), and much information has been gathered over the past 140 years. Although several theories have been formulated, the exact pathogenesis of this disease remains unknown. Recently, the application of advances in immunopathology to the study of aural cholesteatoma has provided a new research angle on cholesteatoma pathogenesis. New knowledge of the immunologic function of the epithelium, specifically of epidermal Langerhans' cells, might enable us to determine the role of the epithelium in cholesteatoma formation. Epidermal Langerhans' cells are thought to be sentinel cells of the cutaneous immune surveillance system (Toews et al. 1980). Several authors (Veldman et al. 1984; Gantz, 1984; Takahashi & Nakano, 1989) have suggested further that the Langerhans' cells within the cholesteatoma matrix were responsible for generating and maintaining the chronic immunological reactions to this disease, while other authors (Kaehoener et al. 1984; Palva & Taskinen, 1987; Aeberg et al. 1988) did not support the hypothesis of Langerhans' cells as having a primary role in the development of cholesteatoma.

The morphology and immunohistochemistry of the newly formed skin in the open-mastoidectomized cavity (cavity skin) was investigated to compare it with that of the cholesteatoma matrix (Park et al. 1993). The cavity skin is definitely newly-formed, but originates in, and migrates via, the ear canal skin even though it is more hyperproliferative than the ear canal skin. The morphology of the cavity skin is much similar to that of cholesteatoma matrix.

The aim of this study was to measure the
occurrence rate of Langerhans' cells in the cholesteatoma matrix, cavity skin, and postauricular skin, and to evaluate their pathological significance. This study will be helpful in understanding the morphology of cholesteatoma and will provide a basis for future immunological studies concerning the pathogenesis of cholesteatoma.

**MATERIALS AND METHODS**

Specimens of the cholesteatoma matrices were collected from 9 patients during cholesteatoma surgery. Among these, 5 cases showed clinical symptom of otorrhea, while the other 4 cases showed no sign of infection. For controls, 6 cavity skins were obtained during tympanomastoid revision surgery which had been done through the open cavity technique; at the same time, 6 normal cutaneous tissues were also obtained from the postauricular incision area of patients.

A portion of each specimen was immediately immersed in 25% cacodylate-buffered glutaraldehyde for electron microscopic examination. They were trimmed and embedded in Epon. Sections 80 to 100nm thick were cut on an ultramicrotome, stained with uranyl acetate and lead citrate, and examined under a Hitachi H-600 electron microscope. The remainder of fresh specimens were incubated in 0.02% EDTA to separate the epidermis from subepithelial tissue, and fixed in acetone. The other specimens were fixed in 10% buffered formalin for 2 days, and were processed as usual. Immunohistochemical staining of acetone-fixed specimens and dewaxed paraffin sections were done using avidin-biotin-peroxidase complex (ABC) technique. Primary antibodies used were anti-mouse I-Ad(Becton Dickinson, Mountain View, California; diluted 1:20) for fresh specimens and rabbit anti-cow S-100 protein (Biomed, Foster City, California) for paraffin sections. All specimens were also stained with hematoxylin and eosin (H&E) for the conventional histological examination.

The I-Ad-positive cells, which were easily identified as dark brown dendritic cells, were counted by means of a reticle fitted into the eyepiece of the microscope and calibrated to a magnification of 400. For each specimen, cells were counted in 7 fields, in which interfollicular areas were selected. The cell populations were expressed as the average number of cells per mm². The statistical analysis was made using Kruskal-Wallis test with a probability value(p) of 0.05. Also, the number of S-100 protein-immunoreactive Langerhans' cells in the suprabaral layer per 1 mm on the basement membrane was calculated for all paraffin-sectioned specimens. The degree of subepithelial inflammation was roughly divided into three grades: mild (+) which was less than 50 inflammatory cells per mm²; moderate (+++) which was 50–200 in-flammatory cells per mm²; and severe (++++) which was over 200 inflammatory cells per mm².

**RESULTS**

The cholesteatoma matrix of varying thickness, from thick to extremely thin, showed keratinized stratified squamous epithelium (Fig. 1). The specimens from subjects with clinical symptom of otorrhea tended to have thicker epithelium, in which many inflammatory cells were found to have invaded the epithelium and subepithelial tissues.

The transmission electron microscopic appearance of Langerhans' cells in the cholesteatoma matrix showed characteristic multilobulated nuclei and long cytoplasmic extension (Fig. 2). At higher magnification, the cytoplasm was agranular but contained mitochondria, golgi apparatus, and Birbeck's granules(Fig. 3). Birbeck's granules are rodlike or tennis racket-shaped structures which are characteristic for Langerhans' cells.

Numerous I-A-positive dendritic Langerhans' cells were identified in the cholesteatoma (Fig. 4), cavity skin (Fig. 5) and postauricular skin (Fig. 6) specimens. Langerhans' cells in the cholesteatoma and cavity skin had more numerous and longer dendritic processes than those in the postauricular skin. The
Fig. 1. Cholesteatoma matrix shows keratinizing stratified squamous epithelium consisting of four distinct layers. Subepithelial tissue is infiltrated by inflammatory cells which are mostly lymphocytes (H&E, ×200).

Fig. 2. Transmission electron microscopic graph shows Langerhans' cell (L) lacking intercellular bridge on its cell membrane and keratinocytes (K). D: desmosome, M: melanin, T: tonofilament, (×10700).
Fig. 3. Higher magnification of Langerhans' cell in Fig. 2 demonstrates characteristic Birbeck's granule (arrow) ($\times$ 60,000).

Fig. 4. I-Ad-positive Langerhans' cells of the cholesteatoma matrix are greater in number and show more numerous and longer dendrites than those in the postauricular skin (DAB, $\times$ 400).
Fig. 5. Langerhans' cells of the cavity skin are greater in number and show more numerous dendrites than those of the cholesteatoma matrix (DAB, ×400).

Fig. 6. I-Ad- positive dendritic Langerhans' cells are noted in the postauricular skin (DAB, ×400).
number of I-Ad-positive dendritic cells in the cholesteatoma and cavity skin was noticeably greater than in postauricular skin; the cavity skin showed the largest population. The median of Langerhans cells in the cholesteatoma, cavity skin and postauricular skin shows in Fig. 7. S-100 protein-positive Langerhans' cells were also found in the epithelium and subepithelial tissue of the cholesteatoma (Fig. 8).

Fig. 7. Distribution of density of I-Ad-positive Langerhans' cells (LC) in cholesteatoma, cavity skin and postauricular skin (p<0.05 as compared to control skin: Kruskal-Wallis test).

Fig. 9. The correlation between the number of Langerhans' cells and the degree of subepithelial inflammation in the cholesteatoma is directly proportional.

Fig. 8. S-100 protein-immunoreactive Langerhans' cells are distributed in the cholesteatoma matrix. They are also found in the subepithelial tissue (DAB, ×200).
8). The more inflammatory cells there were in the subepithelial tissue of the cholesteatoma, the more Langerhans' cells there were. There tended to be a direct correlation between the number of Langerhans' cells and the degree of the subepithelial inflammation in the cholesteatoma (Fig. 9).

**DISCUSSION**

It is now accepted that Langerhans' cells play an important role in the cutaneous immune surveillance system, functioning as antigen presenting cells like macrophages. These dendritic cells constitute 1% to 3% of the epidermal cell population, and have ultrastructurally unique Birbeck granules which are tennis racket-shaped structures. Epidermal Langerhans' cells bear surface receptors, Fc-IgG and C3b, similar to macrophages, suggesting an immunocompetence function. Also, some Langerhans' cells, but not all, express surface la antigen, or class-II MHC glycoprotein (Streilein et al. 1984).

Gantz (1984) hypothesized that the keratin, intracellular debris, and bacteria in the retraction pocket of cholesteatomas might be recognized as nonspecific antigens by epithelial Langerhans' cells, which bound and presented them to lymphocytes either in the epithelium or regional lymph nodes, generating a cytotoxic T-cell response directed at the initial site of sensitization. Even though the specific mechanism of activation is unknown, it appears that Langerhans' cells in the cholesteatoma matrix initiate an immunologic response to the presence of antigen, manifested as an inflammatory reaction. Langerhans' cells in the cholesteatoma matrix could be an important factor in activating and maintaining a chronic inflammatory state in cholesteatomas, resulting in connective tissue breakdown and bone resorption.

Veldman (1985) found an abundant Langerhans' cell population not only in the cholesteatoma matrix but also in the subepithelial tissue. In his opinion, this could be the route for the mobilized Langerhans' cells on their way to the squamous epithelium. Palva et al. (1987) did not agree with Veldman's systemic concept of cholesteatoma. Their observation of Langerhans' cells in the secretory epithelium of subjects with secretory otitis media and chronic otitis media, in which there was not the slightest sign of keratinization or even metaplasia, argues against a specific role for Langerhans' cells in recurrent cholesteatoma. Langerhans' cells must be regarded as normal immune defense cells.

Our study revealed that the presence of Langerhans' cells in the cholesteatoma matrix showed a marked difference in number and shape according to the inflammatory condition of the subepithelial tissue of cholesteatoma. Langerhans' cells in those cases with more inflamed cholesteatoma matrix were more numerous compared to those cases with less-inflamed cholesteatoma matrix. The Langerhans' cells in the cholesteatoma matrix with more inflammation had also more numerous and longer dendritic processes than those in the normal postauricular skin or the cholesteatoma matrix with less inflammation. These morphological findings suggest an activated condition of Langerhans' cells in the inflammatory cholesteatoma matrix. Such an increased number of Langerhans' cells and elongation of dendritic processes have also been observed in the cavity skin. It was very interesting that the number of Langerhans' cells in the cavity skin was greater than that in the cholesteatoma matrix. This finding could mean that the cavity skin was more infected than the cholesteatoma matrix. That was why we did the revision tympanomastoidectomy to remove the infected cavity skin. Langerhans' cells were found in our present study to be scattered in the subepithelial connective tissue of both the cholesteatoma matrix and the cavity skin, especially in the cases with more inflammation. This agrees with a previous study showing an intimate relation between Langerhans' cells and lymphocytes in the subepithelial tissue of cholesteatomas (Veldman, 1985). Under inflammatory skin conditions, it has frequently been observed that Langerhans' cells are in contact with lymphocytes in the dermis. These
Langerhans' cells are believed to function as antigen-presenting cells at these sites (Toews et al. 1980).

The marked difference in numbers and shapes of Langerhans cells' according to the inflammatory condition of the cholesteatoma, and the colocalization of Langerhans' cells with lymphocytes in the subepithelial tissue of the cholesteatoma, suggest that these cells might play an important role in the immunodefense system. However, such findings of Langerhans' cells in the cholesteatoma were also observed in the cavity skin. Therefore, we agree with Palva and Taskinen (1987) that Langerhans' cells must be regarded as normal immune defense cells, and nonspecific to the development of cholesteatoma. The results of our study do not support the hypothesis that Langerhans' cell have a primary role in the development of the cholesteatoma; however, their significant presence in inflammatory sites suggests that these cells may be involved in the proliferation of existing cholesteatoma although inflammatory reactions and bacterial infections may aggravate an existing cholesteatoma proliferation. Thus, the significance and nature of the role of Langerhans' cells in the development of cholesteatomas requires further study.

REFERENCES