Langerhans Cells in the Larynx and the Hypopharynx

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Summary: Langerhans cells are important in the human immune system as a part of the dendritic cell system. This study was carried out to demonstrate the presence of Langerhans cells and their distribution in normal human larynx and hypopharynx, by light (S-100 polyclonal antibody) and transmission electron microscopy. The results are summarized as follows: 1) Langerhans cells with cytoplasmic granules (Langerhans granules or Birbeck's granules) were situated in the suprabasal region of the squamous epithelium in the larynx and the hypopharynx. 2) They distributed in the membranous portion of the vocal folds, epiglottis, aryepiglottic folds, arytenoid regions and interarytenoid notch of the larynx. The cells surrounded the vestibule and glottis of the larynx. 3) They distributed in the postcricoid area, piriform sinus and posterior pharyngeal wall of the hypopharynx. The cells were situated above the entrance of esophagus. 4) The cells were suggested to be a factor of immune response at the entrance of trachea and esophagus.

Key words Langerhans cell, immunology of larynx and hypopharynx, histology of larynx and hypopharynx, vocal folds, larynx, hypopharynx

Introduction

Langerhans cells are important in the human immune system as a part of the dendritic cell system (Romani and Schuler, 1992). Dendritic cells are a system of antigen presenting cells and have an ability to capture antigens and initiate T cell-mediated immunity (Steinman, 1991).

Schenk (1980) demonstrated the presence of Langerhans cells in squamous cell carcinoma of the larynx identified by ultrstructural examination of

cytoplasmic granules (Langerhans granules or Birbeck's granules). Gallo et al. (1991) noted the presence of Langerhans cells in squamous cell carcinoma of the larynx using the polyclonal antibody against S-100 protein. Thompson and Griffin (1994) noted Langerhans cells in normal and pathological vocal fold mucosa, using the S-100 polyclonal antibody.

Demonstration of the presence and distribution of Langerhans cells in normal human larynx and hypopharynx

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is but little reported. The purposes of this study were to demonstrate the presence of Langerhans cells and their distribution in the normal human larynx and hypopharynx.

Materials and Methods

Seven normal human larynges and hypopharynges obtained from autopsy cases were used. Four of these were from men with ages ranging from 45 to 56, and 3 from women with ages ranging from 43 to 58. They didn't suffer from diseases of larynx and hypopharynx and immunologic disorder.

Langerhans cells can be detected histologically in formalin fixed and paraffin embedded tissue expression S-100 protein on immunohistochemistry. For the present immunohistochemistry study, 4 specimens were fixed in 10% formalin. Serial sections were made. They were dehydrated in graded concentrations of ethanol, embedded in paraffin and stained with S-100 polyclonal antibody (DAKO corporation, USA, 1:500) using a labeled streptavidin biotin (LSAB) technique (DAKO LSAB kit).

For the transmission electron microscopic study, three specimens were fixed in 2.5% glutaraldehyde at 4 °C for 2 hs. After being rinsed with cacodylate buffer solution, they were postfixed in 2% osmium tetroxide with Millonig's phosphate buffer solution at 4 °C for 2 hs. This was followed by dehydration in graded concentrations of ethanol and embedding in epoxy resin. Semithin sections were made with an ultramicrotome, stained with 1% toluidine blue and examined by light microscopy. Thin sections were made with an ultramicro-

tome, stained with uranyl acetate and lead citrate and observed under a JEM-2000EX transmission electron microscope.

Results

Morphologic findings of the Langerhans cells in the larynx and hypopharynx

Figures 1A and 1B show light microscopic findings of Langerhans cells in the larynx and the hypopharynx. They were present in squamous epithelium in the larynx and the hypopharynx, as shown using the S-100 polyclonal antibody. They were dendritic in shape (Figs 1A and B) and situated in the suprabasal region of the squamous epithelium (Fig. 1A).

Figures 1C-E show transmission electron microscopic findings of Langerhans cells. They were dendritic in shape. They possessed clear cytoplasm (Figs 1C and D) which contained no tonofilaments (Figs 1C and D). Desmosomes were absent between keratinocytes (Figs 1C and D). The nuclei were markedly folded and the nucleoplasm was relatively clear (Fig. 1D). Golgi apparatus, mitochondria and rough endoplasmic reticulum were seen in the cytoplasm. Some cytoplasmic granules (Langerhans granules or Birbeck's granules) showing rod-shaped and median striated lines in the cytoplasm could be seen (Fig. 1E). The size of these granules was approximately 40 nm in width and 200 nm in length. Occasionally lysosomes containing melanin granules were present in Langerhans cells (Fig. 1D).

Distribution of the Langerhans cells in the larynx and the hypopharynx

In the larynx, the Langerhans cells

Fig. 1A. Langerhans cells in the squamous epithelium at arytenoid of the larynx. (S-100 antibody stain, original $\times 100$) arrow: darkly staining Langerhans cells, SE: squamous epithelium, LP: lamina propria

Fig. 1B. Langerhans cells in the squamous epithelium at piriform sinus of the hypopharynx. (S-100 antibody stain, original $\times 400$) arrow: darkly staining Langerhans cells.

Fig. 1C. Transmission electron microscopic (TEM) findings of Langerhans cells in the squamous epithelium at postcricoid area of the hypopharynx. arrow: Langerhans cells, Kc: keratinocyte

Fig. 1D. TEM findings of Langerhans cells in the squamous epithelium at vocal fold of the larynx. arrow: Langerhans cells, Ly: lysosomes, N: nucleus, Kc: keratinocyte

Fig. 1E. TEM findings of cytoplasmic granules in Langerhans cells. arrow: cytoplasmic granules (Birbeck's granules).

were present in squamous epithelium in the epiglottis, aryepiglottic folds, arytenoid regions, interarytenoid notch and membranous portion of the vocal folds. They surrounded the vestibule and glottis of the larynx. In the hypopharynx, they were situated in squamous epithelium in the postcricoid area, piriform sinus and posterior pharyngeal wall of the hypopharynx.

Discussion

Langerhans cells are important component of the human immune system as a part of the dendritic cell system. Dendritic cells constitute a system of

antigen-presenting cells widely distributed throughout the body and have a outstanding capacity to initiate primary immune response (Romani and Schuler, 1992). They acquire antigens in the tissues and process them in an immunogenic form (Romani and Schuler, 1992). They convey immunogens to lymphoid organs and detect and efficiently activate antigen-specific T cell clones to generate immune response (Romani and Schuler, 1992). Dendritic cells comprise a system that occupies discrete portions of non-lymphoid and lymphoid organs (Steinman, 1991). Langerhans and interstitial dendritic cells constitute the dendritic cell system in nonlymphoid organs (Steinman, 1991).

Langerhans cells characterized by the presence of cytoplasmic granules (Langerhans granules or Birbeck's granules) have been detected not only in the epidermis (Birbeck et al. 1961; Breathnach, 1964) but nose (Jahnke, 1974), oral cavity (Schroeder and Theilade, 1966) and cervix uteri (Hackermann et al. 1968) of humans. Langerhans cells are present under both normal as well as pathological conditions (Hammar et al. 1986). Regarding the larynx, Schenk (1980) observed Langerhans cells in squamous cell carcinoma of the larynx, identified by ultrastructures of cytoplasmic Birbeck's granules. Gallo et al. (1991) noted Langerhans cells in the squamous cell carcinoma of the larynx using the polyclonal antibody against S-100 protein, concluded that the presence of high or intermediate density of Langerhans cells and of marked lymphoid inflammation may be considered favorable prognostic factors for patients with squamous cell carcinoma of the larynx. Thompson and Griffin (1994) reported Langerhans cells in normal and pathological vocal folds, using the S-100 polyclonal antibody. Regarding the hypopharynx, Langerhans cells in it were little reported. Demonstration of the presence and distribution of Langerhans cells in normal human larynx and hypopharynx remain unclear. Langerhans cells were shown in this study to be characterized by the presence of cytoplasmic granules (Langerhans or Birbeck's granules) using an electron microscope in normal human larynx and hypopharynx; their distribution was also clarified.

There are immunological resistance of mucosa, i.e., local, humoral and cellular immunity. Mogi et al. (1979)

studied the biological properties of laryngeal secretion and found laryngeal secretions to contain IgG, IgA, IgE, secretory components and lactoferrin. They emphasize the importance of the local immune system by secretory IgA in the laryngeal mucosa. Humoral immunity, components are immunoglobulins present throughout the body, is in the larynx. Langerhans cells presented in this study, macrophage and T cells are important component of cellular immunity. Brandtzaeg (1995) reported three principles involved in defense of airway mucosa. 1. Immune exclusion which limits epithelial colonization of microorganisms and inhibits the entrance of foreign material. Locally produced secretory IgA and secretory IgM, and IgG antibodies that seep through the epithelium are responsible for immune exclusion. 2. Immune regulation which requires interactions between antigen presenting cells (dendritic cells, macrophages), T cells, and B cells. 3. Immune elimination for the neutralization and elimination of foreign material that has penetrated through the epithelium. This second line of defense is triggered by cytokines, activated T cells and antibodies. Langerhans cells in the larynx and the hypopharynx may possibly be essential to immune regulation required for defense of mucosa of the airway and the passageway of food.

There may be such a thing as laryngeal allergy. Williams (1972) reported allergic laryngitis. Langerhans cells in the larynx and the hypopharynx may participate in allergic reaction as antigen presenting cells.

This study indicated Langerhans cells to be distributed in squamous epithelium

in the epiglottis, aryepiglottic folds, arytenoids, interarytenoid notch and membranous portion of the vocal fold. They surround the vestibule and glottis of the larynx. They were suggested to be essential to immune response at the entrance of trachea. Basic functions of the larynx are acting as a protective sphincter, passageway for air and sound-production. The larynx is suggested to be essential for immune response in upper respiratory passages. This study also indicated Langerhans cells to be situated in squamous epithelium in the postcricoid area, piriform sinus and posterior pharyngeal wall of the hypopharynx. They were suggested to be essential to immune response above the entrance of esophagus in upper digestive system.

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