An Immunohistochemical and Electron-Microscopic Study of Vascular Endothelial Cells in Vocal Fold Polyps*

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Summary. Gelatinous and telangiectatic types can be differentiated among the human vocal fold polyps. Telangiectatic polyps are characterized by eosinophilic deposits consisting of fibrin and cellular blood constituents. Labyrinthine vascular channels are characteristic of these polyps, and are partially or completely lined by a single layer of flat cells. Using electron microscopy and immunohistochemical stainings (antibodies against factor VIII-related antigen, Ulex europaeus I lectin, and antibodies against lysozyme), we found that the lining cells are true vascular endothelial cells and are not organizing histiocytic cells that are arranged in an endothelial-like pattern.

Key words: Vocal fold polyps – Endothelial cell – Immunohistochemistry – Electron microscopy

Introduction

Vocal fold polyps are common benign lesions of the larynx. Two different types of polyps can be differentiated histologically. Gelatinous polyps have an edematous stroma and a basophilic appearance in their central parts. Telangiectatic polyps are characterized by a labyrinthine network of ramifying sinus-like spaces between which are located eosinophilic deposits [6, 12]. Using conventional staining techniques and light microscopy, the nature of the cells lining these spaces has remained obscure. In the present immunohistochemical and electron microscopic study we have been able to show that these lining cells are true endothelial cells.

* Professor Dr. W. Hort to his 60th birthday

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Materials and Methods

Human vocal fold polyps were removed from symptomatic patients by endolaryngeal microsurgery, and tissues were immediately fixed in 4% formalin and embedded in paraffin. Then 4-μm-thick paraffin sections were stained with hematoxylin-eosin, van Gieson elastic stain, Berlin blue iron stain, and phosphotungstic acid-hematoxylin and were examined under light microscopy. Fresh tissue samples were separately fixed in 2.25% cacodylate-buffered glutaraldehyde for electron microscopy. These tissues were dehydrated in a graded ethanol series and embedded in Epon. Ultrathin sections were stained with uranyl acetate and lead citrate, and were examined with a Zeiss electron microscope (EM 109).

Immunohistochemistry

Paraffin sections were treated with polyvalent antibodies against factor VIII-related antigen and lysozyme (Dakopatts, Copenhagen, Denmark) according to the peroxidase-antiperoxidase (PAP) method of Sternberger et al. [18]. Deparaffinized sections were preincubated with 0.3% H2O2 in methanol and 0.0125% trypsin for 40 min. Tissues were next incubated with diluted normal mouse IgG (Sigma Chemicals, Munich, FRG), following which primary rabbit anti-human antibodies, secondary swine anti-rabbit IgG and a peroxidase-rabbit-antiperoxidase complex (Dakopatts) were sequentially applied. The reagents were used in appropriate dilutions in Tris buffer. The site of antibody binding was visualized with 3,3-diaminobenzidine (Sigma Chemicals). Further serial sections were treated with biotiniolated Ulex europaeus I lectin (UEA I, Vectastain, Vector Laboratories, Burlingame, California, USA) according to Weber et al. [20]. To this substance peroxidase is linked by the ABC-complex (Vector Laboratories).

Results

Light Microscopy

Telangiectatic polyps were seen to be covered by a stratified non-keratinizing squamous epithelium and were composed of large eosinophilic deposits, irregular cavities, and arterial and venous blood vessels. The eosinophilic deposits consist of erythrocytes, blood platelets, fibrin, and plasma proteins. The cavities between these deposits have an irregular sinusoidal appearance and are incompletely lined by a single layer of flat cells, which have slightly prominent nuclear regions. In addition to a small number of lymphocytes and granulocytes, the eosinophilic deposits contain histiocytic cells with foamy cytoplasm and plasmatic inclusions (Fig. 1a–d).

Immunohistochemistry

Factor VIII-Related Antigen. The endothelial cells of blood vessels and the cell linings of the sinus-like spaces present are stained with equal intensity. The cells inside the deposits are devoid of a reaction product, similar to the squamous epithelium (Fig. 2a).

Ulex Europaeus I Lectin. A positive reaction product is present in the vascular endothelial cells and in the cells lining the sinus-like spaces, and contrasts with the negative reaction of the cells dispersed in the eosinophilic deposits. Strong coloration is also demonstrable in the epithelial cells of the epidermal cover and in the erythrocytes (Fig. 2b).