IMMUNOCYTOCHEMICAL DETECTION OF ENDOTHELIN DURING THE DEVELOPMENT OF MURINE LUNG

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Aim. In the respiratory tract of mammals several roles of the peptide endothelin (ET) -vasoactive effects, constriction of bronchial smooth muscle, proliferation of several cell types- have been indicated. In development it has been suggested that ET may participate in the maintenance of high foetal pulmonary vascular resistance and in the control of vascular tone in neonates. Although there is a general agreement about the production of endothelin by pulmonary epithelial cells, it is not well established which epithelial cell type/s is/are involved in the genesis of ET. The present study focus in the detection of ET-immunoreactive cells in murine lung during adulthood and development.

Materials and Methods. The immunocytochemical method of avidin-biotin complexes using several antisera raised against ET and its precursor, big-ET have been applied to paraffin-embedded lungs sections. Mice foetuses (E-gestational-days 13, 14, 15, 16, 17, 18, and 19), postnatal -P- (0, 1, 2, 6 days) and adult (ad) animals were studied.

Results. Immunoreactivity for ET-1 and big-ET has been obtained in most of epithelial (Clara) cells of both extrapulmonary bronchi and intrapulmonary bronchioles. No labelling has been observed in ciliated, endocrine or alveolar cells. Immunolabeling has been detected for the first time in the bronchi and large bronchioli of E-18 foetal mice (Fig. 1) and appears later in lower airways. As development continues, the apical, faint immunoreactivity increases in intensity and extends towards more basal cytoplasmic regions (Figs. 2, 3). Although, as indicated, they appear later in small airways, the proportion of positive cells in mature lungs is higher in small bronchioles than in larger airways (Fig. 2). Results are summarized in the following table.

	High airways	Low airways
E-18	+ (only apical)	-
P-0	+ (mainly apical)	+ (apical)
P-6	+ (apical, basal)	+ (apical, basal)
P-ad	+ (apical, basal)	+ (apical, basal)

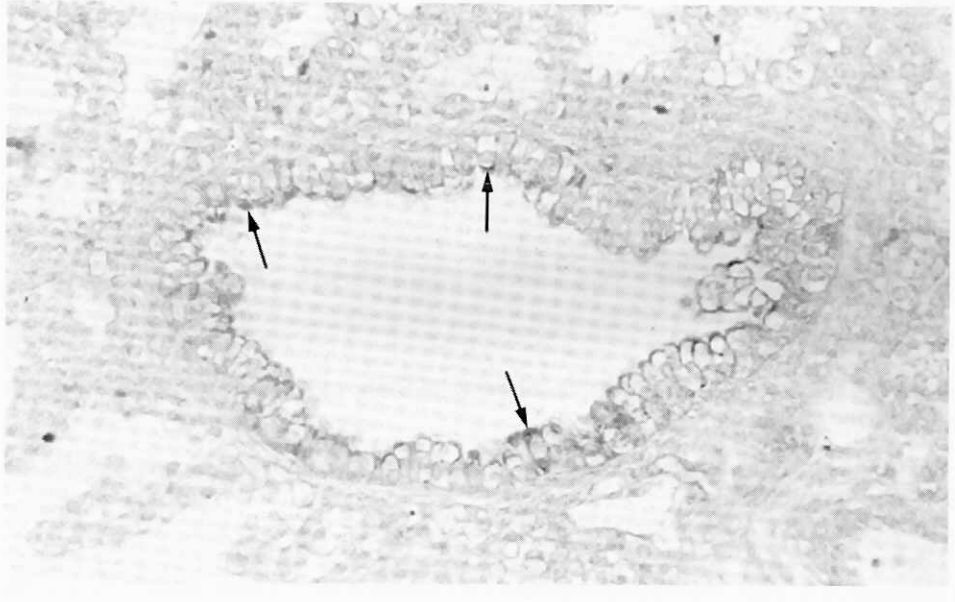

Fig. 1. E-18 mouse lung. Large bronchiole. A faint ET-immunoreactivity is present at the apical cytoplasm of the epithelium (arrows). X 400.

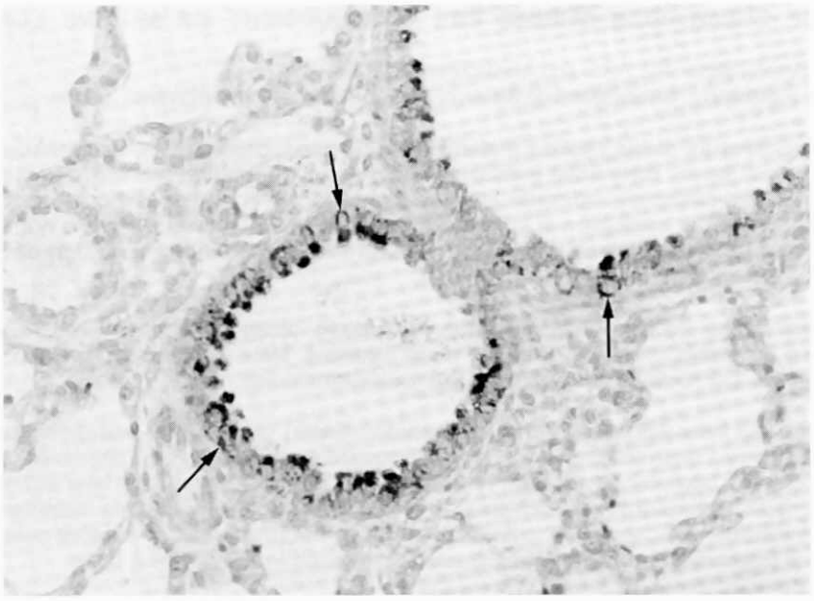

Fig. 2. P-6 lung. Small and medium-sized bronchioles. The immunolabelling extends throughout the cytoplasm. (arrows). Note that the proportion of positive cells is higher in the small bronchiole. X 400.

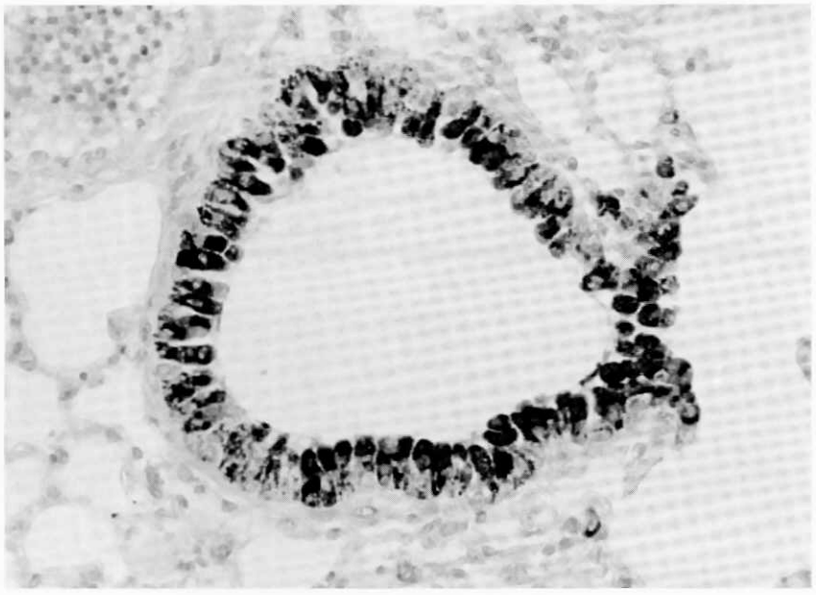

Fig. 3. Ad-lung. Numerous Clara cells with strong immunoreactivity throughout the cytoplasm are present in the bronchiole. X 400.

Conclusions.

1. ET-immunoreactivity is detected from late gestation onwards in Clara cells of bronchi and bronchioli. No other positive epithelial cell types have been found.

2. Since the amount of immunolabelling in airway epithelial lining increases along developoment, the role of ET seems not to be specific of perinatal murine lung phisiology.

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