Original Articles

The Epithelial Framework of the Thymus in Normal and Pathological Conditions * **

Immunohistochemical Demonstration of Keratin in an Autopsy Series

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Summary. Autopsy specimens of normal human thymus, from cases of accidental involution, follicular hyperplasia, thymomas and a teratoma were investigated by immunocytochemistry using specific immune sera to small and large keratins. Keratin antisera represent a "marker" of both Hassall's corpuscles (HC) and so-called epithelial reticular cells. There were no apparent differences in keratin polypeptides distribution between cortical and medullary thymic epithelial cells. In accidental involution, the epithelial framework became prominent: epithelial cortical borders and epithelial perivascular sheaths appeared often to be discontinuous structures. The central and occasionally cystic spaces of HC did not react with keratin antisera. In follicular hyperplasia, almost solid epithelial aggregates were seen which were located around germinal centers. In thymic tumours, neoplastic epithelial cells displayed a marked immunoreactivity with keratin antisera. Immune sera against keratin filaments represent an interesting tool in thymus research and in the diagnostic pathology of thymic tumours.

Key words: Keratin – Filaments – Epithelium – Thymus – Tumour diagnosis

Introduction

The framework of the thymic lobule is formed by a three-dimensional meshwork of epithelial cells of entodermal origin (Sainte-Marie 1974; Oláh et al. 1975; Weiss 1977). In the medulla, the epithelial character of the thymus is most evident from the presence of Hassall's corpuscles (HC) (Kohnen and Weiss 1964; Bargmann 1967). Until now most work on the structure of the thymus has been done by conventional light and electron microscopical methods (Weiss ** This study was supported by a grant from the Deutsche Forschungsgemeinschaft (Lo 285/2-1; Ot 53/4-7)

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Epithelial cells were usually recognized at the ultrastructural level by demonstration of the presence of tonofilaments and desmosomes (Oláh et al. 1975). Tonofilaments are intermediate filaments (8–12 nm in diameter) and consist of various keratin polypeptide subunits (molecular weight: 40,000–67,000 daltons) (Sun and Green 1978; Franke et al. 1978).

Recently, specific antisera against purified keratins were produced in guinea pigs (Viac et al. 1980; Viac et al. 1980). Using these antisera, normal and neoplastic epithelium-derived cells of different organs were specifically labelled (Lönig et al. 1980; Caselitz et al. 1980). Hence, these antibodies represent an appropriate tool with which to analyze the epithelial network in normal and diseased thymuses. The aim of this study was to look for

1. relationships of the keratinization process in skin and thymus,
2. normal organization of epithelial cells in the thymus,
3. changes of epithelial cell arrangement in involuted and hyperplastic thymuses,
4. reliability of immunoenzymatic keratin staining as a rapid light microscopic method to determine epithelium-derived tumours.

For this investigation, two different keratin antisera were used. In previous studies (Viac et al. 1980; Lönig et al. 1980), antibodies against the keratin polypeptide of molecular weight of 67,000 daltons (67 K) were shown to label only the suprabasal layers of the epidermis. Immune sera against the keratin polypeptide of molecular weight of 55,000 daltons (55 K), however, labelled the entire epidermis. Using these two antisera, 20 autopsy cases of fetal, infantile and adult thymuses were investigated by the indirect immunoperoxidase method.

**Materials and Methods**

*Tissue Sampling.* 16 autopsy cases of fetuses, newborns and infants and 4 adults were selected for this investigation. The clinical and pathological data are listed in Table 1. The thymuses were cut into small pieces and fixed in Bouin's solution for 4–8 h. After fixation, the material was dehydrated and embedded in paraffin.

*Immunocytochemistry.* The purification of keratin antigens and the production of specific antibodies in guinea pigs have been published elsewhere (Viac et al. 1980; Viac et al. 1980). For the demonstration of keratin antigens (67 K and 55 K), the indirect immunoperoxidase technique was performed as described in detail in previous publications (Lönig et al. 1977, 1980). Keratin antisera diluted at 1/200 in phosphate buffered saline and a peroxidase-conjugated goat anti-guinea pig immunoglobulin serum (Nordic) diluted at 1/50 were used. The peroxidase activity was demonstrated by 3',3'-diaminobenzidine (Sigma). Control experiments were done by replacing the specific keratin antibodies by the guinea pig pre-immune serum and/or by omitting the primary specific antiserum. Parallel sections were stained by haematoxylin-eosin.

**Results**

Normal Thymus (Figs. 1A–C, 2A, B). Epithelial cells of both thymic cortex and medulla expressed 67K- and 55K-antigenic sites. There were no apparent differences in the distribution of these two keratin polypeptides. In the cortex, markedly stained epithelial cells were arranged in a thin layer at the surface whereas they assumed stellate elongated forms inside the lobule (Fig. 1C). Strongly labelled long, slender epithelial cytoplasmic processes surrounded and