Virchows Arch [Pathol Anat] (1981) 392:7–20

Virchows Archiv A

Original Articles

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

Immunohistochemical Demonstration of Keratin in an Autopsy Series

Th. Löning, J. Caselitz, and H.F. Otto

Institute of Pathology, University of Hamburg (Director: Prof. Dr. G. Seifert), Hamburg, Federal Republic of Germany

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 discontinous 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 immunorectivity 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 corpuscules (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

* Dedicated to Professor Dr. G. Seifert on the occasion of his 60th birthday

** This study was supported by a grant from the Deutsche Forschungsgemeinschaft (Lo 285/2-1; Ot 53/4-7)

Offprint requests to: Dr. Thomas Löning, Institut für Pathologie, Universität Hamburg, Martinistraße 52, D-2000 Hamburg, Federal Republic of Germany

0340-1227/81/0392/0007/$02.80

1977). 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 (Löning 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 microscopical method to determine epithelium-derived tumours.

For this investigation, two different keratin antisera were used. In previous studies (Viac et al. 1980; Löning 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 (Löning 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

Table 1. Human thymuses

Age	Sex	Cause of death	Thymus Morphology
36-week-old fetus	female	amniotic fluid aspiration	normal
38-week-old fetus	male	placental insufficiency	normal
newborn (died after 35 min)	male	internal hydrocephalus	normal
newborn (died after 1 day)	male	immaturity	normal
$2^1/_2$ months	male	internal hydrocephalus	normal
15 months	male	aganglionosis, respiratory insufficiency	normal
7 years	male	accident, cerebral contusion	normal
7 days	male	congenital heart disease	involution
8 days	male	congenital heart disease	involution
18 days	female	subarachnoid bleeding	involution
19 days	male	sinus thrombosis	involution
25 days	female	respiratory distress syndrome	involution
2 months	female	congenital heart disease	involution
$1^1/_2$ years	female	epilepsy, central dysregulation	involution
3 years	male	necrotizing colitis	involution
63 years	male	myocardial infarction	hyperplasia
newborn (died after 2 h)	male	respiratory distress syndrome	adult teratoma
57 years	male	cardiorespiratory insufficiency	lymphoepithelial thymoma
73 years	male	acute pancreatitis	epithelial thymoma
77 years	female	ovarian carcinoma	lymphoepithelial thymoma

enclosed thymocytes. The blood vessels inside the lobule were separated by a labelled epithelial sheath from the lymphoid tissue (Fig. 2A, B). This epithelial covering of cortical and medullary vessels, however, was often discontinous as seen by immunohistochemistry (Fig. 2A). In the medulla, the epithelial cells were packed more closely and often showed a polygonal rather than elongated appearance (Fig. 2A). HC were strongly labelled by both keratin antisera. The central spaces of HC, however, displayed a patchy or even absent staining (Fig. 1B, 2A). In this interior area, cellular debris was seen intermingled with inflammatory cells, particularly granulocytes. Keratin antisera never labelled the lymphoid tissue and the perithymic mesenchymal structures.

Accidental Involution (Figs. 3A–C, 4A, B). The main characteristic of involuted thymuses was the low content of non-labelled cellular elements in the thymic lobules (Fig. 3B, C). Few lymphocytes and macrophages were seen within the heavily labelled and closely packed epithelial reticulum. There was no sharp delineation between cortex and medulla (Fig. 3A, B). HC showed an occasionally cystic appearance (Fig. 4A). These cystic spaces were filled with an amorphous material including nuclear dust and granulocytic cells which showed no staining with keratin antibodies (Fig. 4A, B).

Follicular Hyperplasia (Fig. 5A–C). In thymus follicular hyperplasia, large completely negative germinal centers were surrounded by strongly labelled epithelial

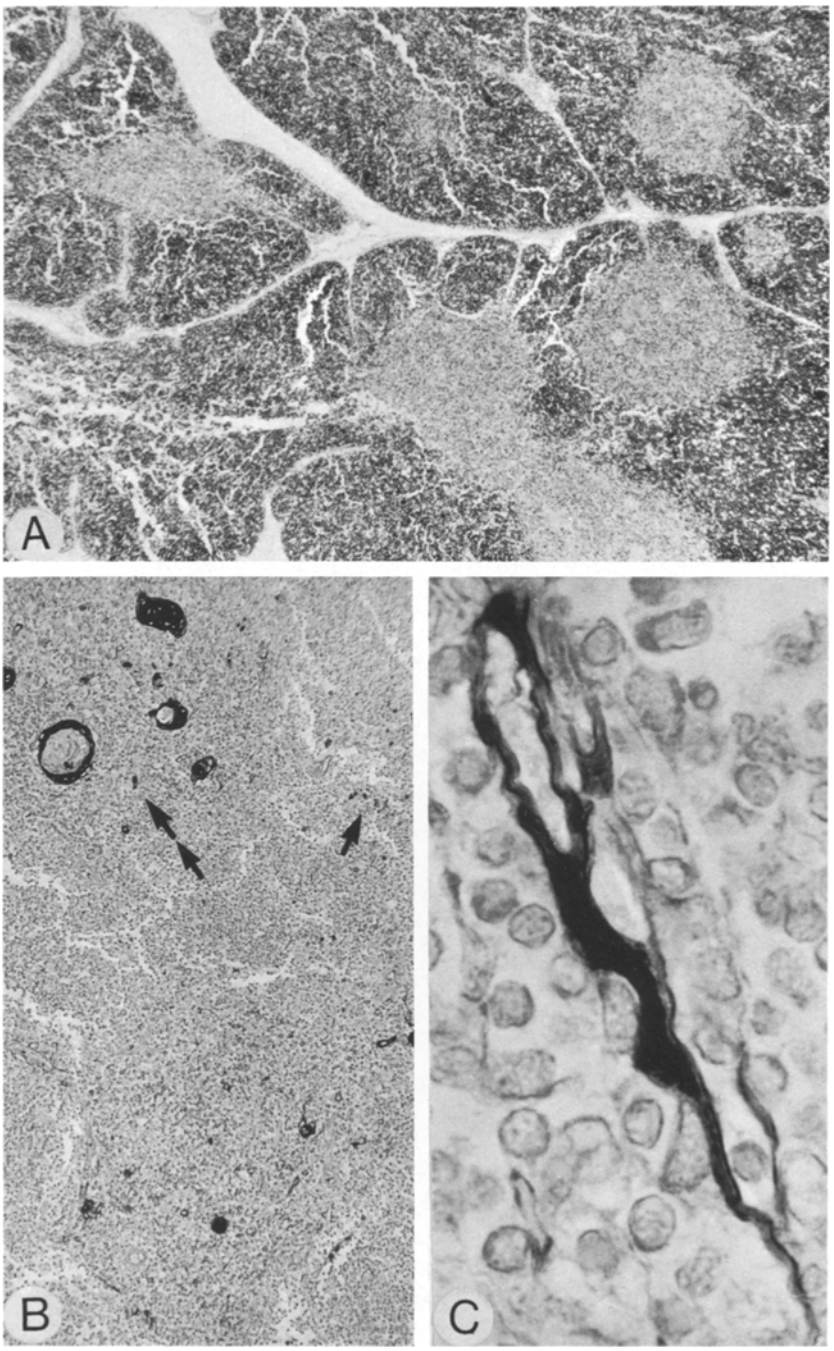

Fig. 1A–C. **A** Cortico-medullary architecture of thymic lobules. Haematoxylin-Eosin. Mag. ×90. **B** Keratin staining. Positive HC and epithelial reticular cells in cortex (arrow) and medulla (double arrow). Indirect immunoperoxidase method. No counterstaining. Mag. ×90. **C** Positive elongated epithelial reticular cell. Negative mesenchymal cells. Indirect immunoperoxidase method. No counterstaining. Mag. ×1,370

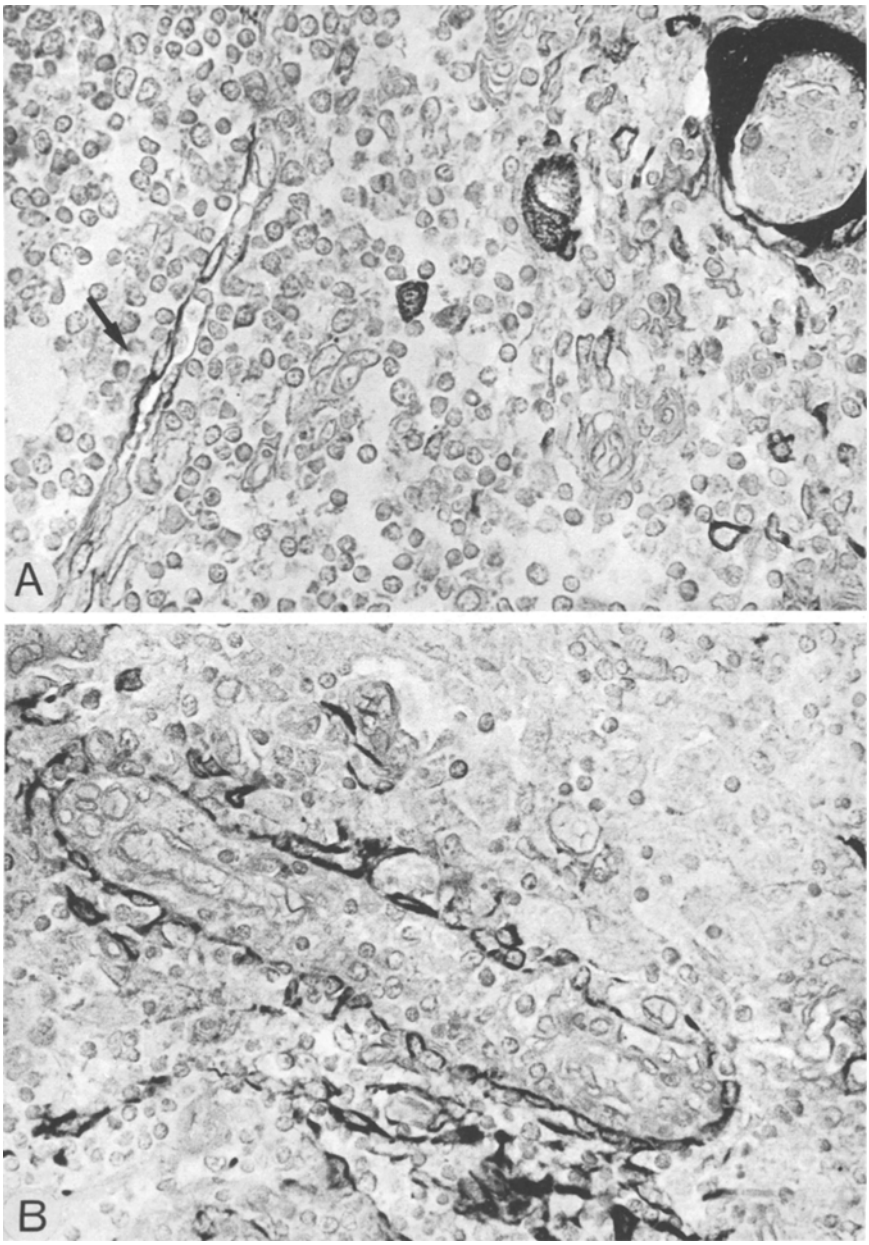

Fig. 2A, B. Normal thymus. Demonstration of epithelial perivascular sheaths. **A** Small venule in the medulla (arrow). Dyscontinous epithelial barrier. In the right upper corner HC with a negative central space. Indirect immunoperoxidase method. No counterstaining. Mag. ×550. **B** Small vessel in the cortex. Aspect of a more closely arranged epithelial sheath. Indirect immunoperoxidase method. No counterstaining. Mag. ×550

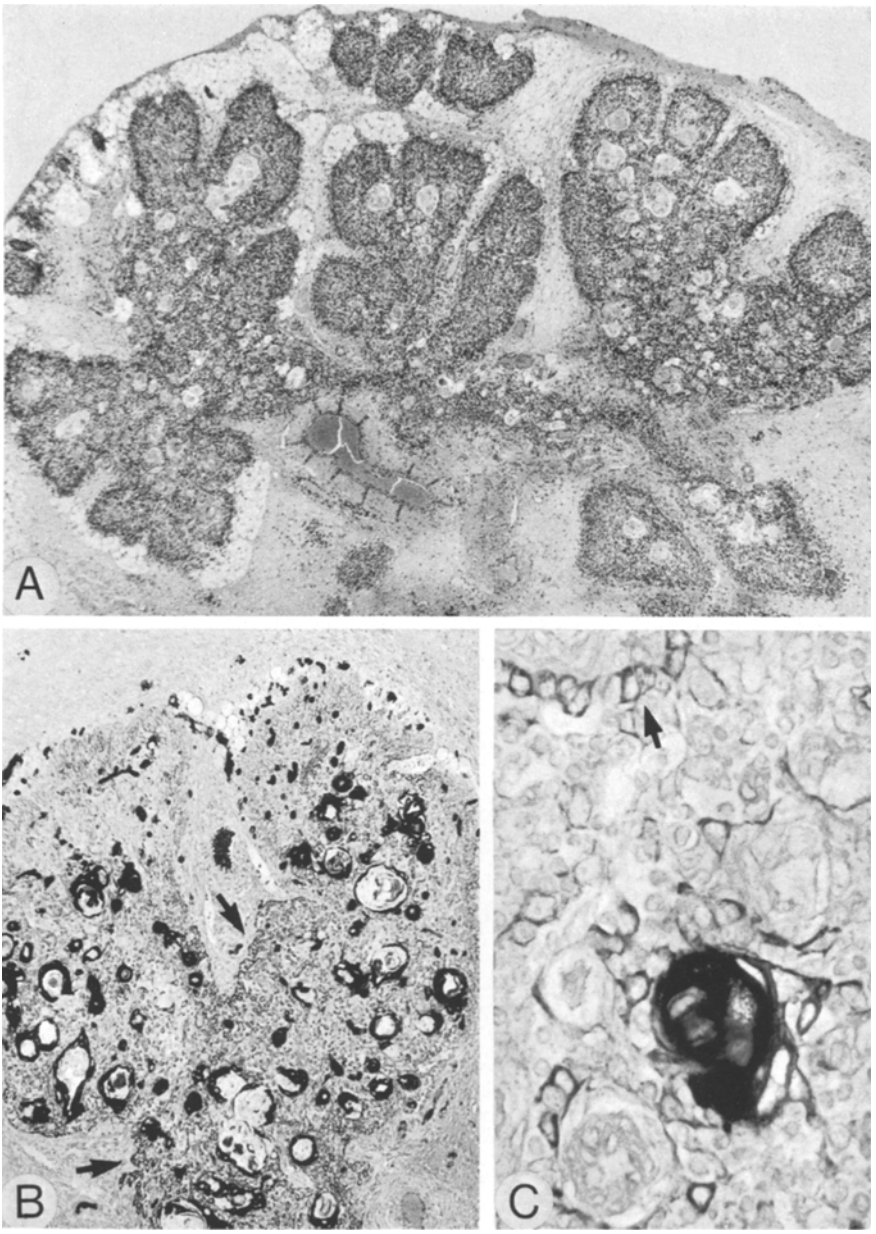

Fig. 3A–C. Accidental involution. **A** Prominent epithelial framework. Depletion of lymphocytes. Haematoxylin-Eosin. Mag. × 35. **B** Keratin staining. Strong labelling of HC, cortical and medullary epithelial reticular cells. Demonstration of cortical borders (arrows). Indirect immunoperoxidase method. No counterstaining. Mag. ×90. **C** Higher magnification of cortical border (arrow) and epithelial network including HC. Indirect immunoperoxidase method. No counterstaining. Mag. × 550

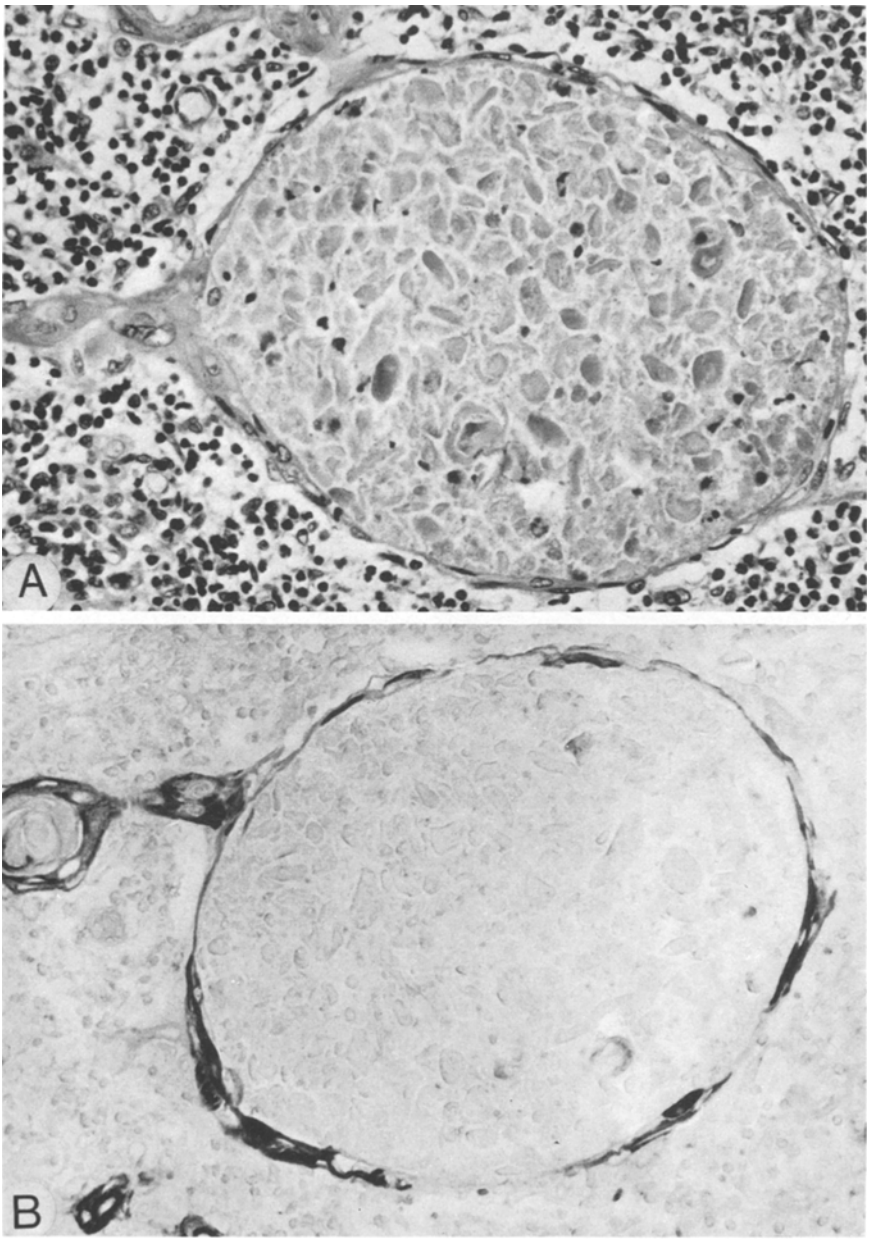

Fig. 4A, B. Accidental involution. Aspect of cystic HC. **A** Conventional staining. Central space is filled with amorphous material including nuclear dust. Haematoxylin-Eosin. Mag. $\times 340$. **B** Keratin staining of a parallel section. Negative central space. Indirect immunoperoxidase method. No counterstaining. Mag. $\times 340$

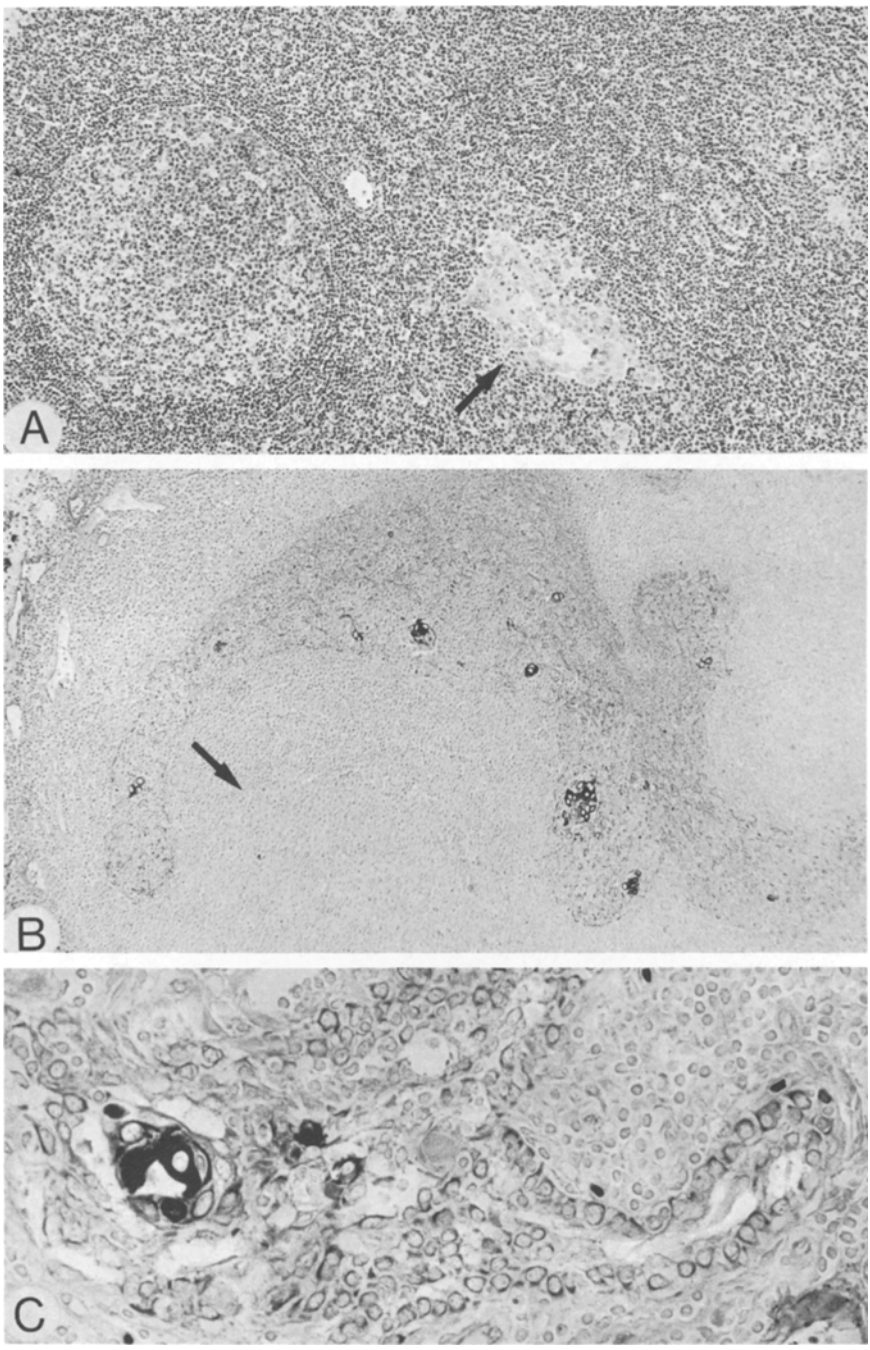

Fig. 5A–C. Follicular hyperplasia. **A** Germinal center formation. In the neighbourhood epithelial aggregate (arrow). Haematoxylin-Eosin. Mag. ×90. **B** Keratin staining. Negative germinal center (arrow). Nearly solid epithelial complexes including HC around the germinal center. Indirect immunoperoxidase method. No counterstaining. Mag. ×90. **C** Higher magnification of strongly positive epithelial aggregate. Indirect immunoperoxidase method. No counterstaining. Mag. ×550

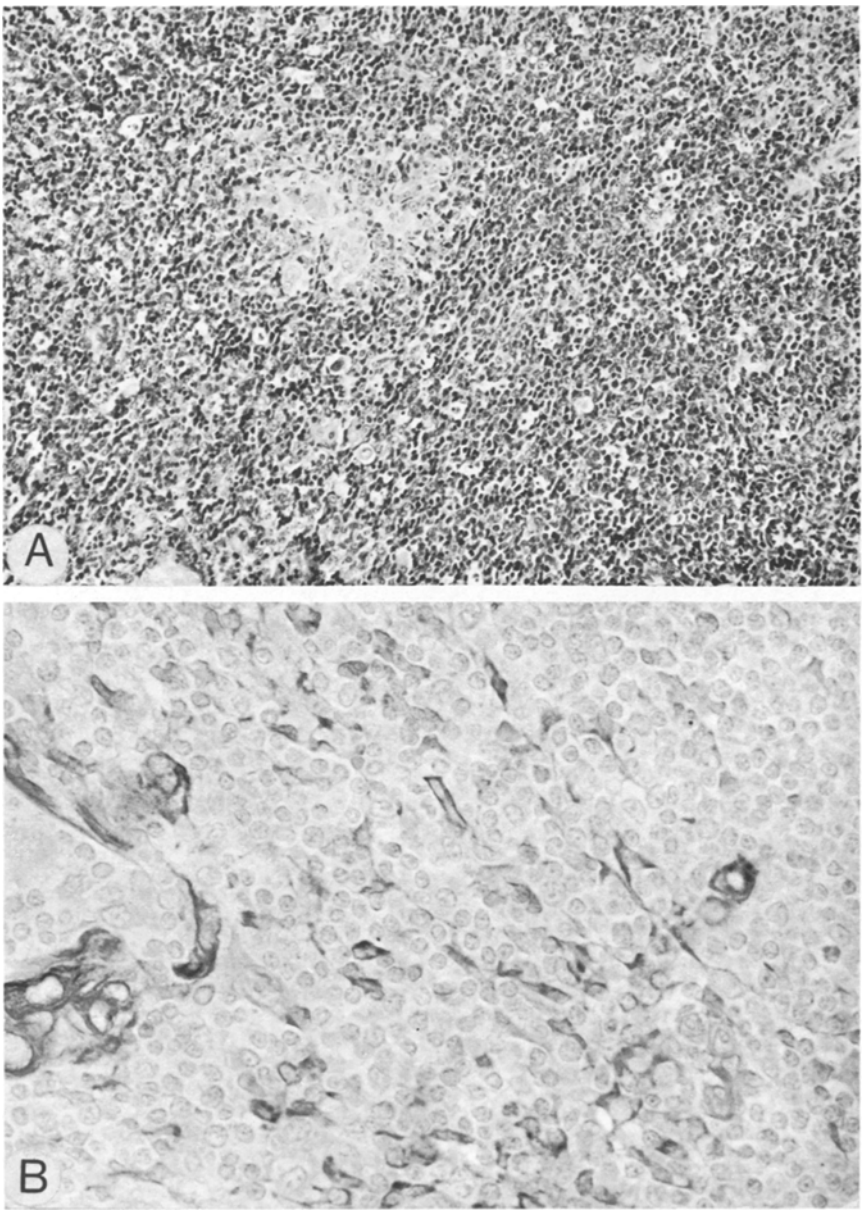

Fig. 6A, B. Lymphoepithelial thymoma. **A** Conventional staining of the tumour. Haematoxylin-Eosin. Mag. ×140. **B** Keratin staining of a parallel section. Positive neoplastic epithelial cells. Lymphocytes are negative. Indirect immunoperoxidase method. No counterstaining. Mag. ×340

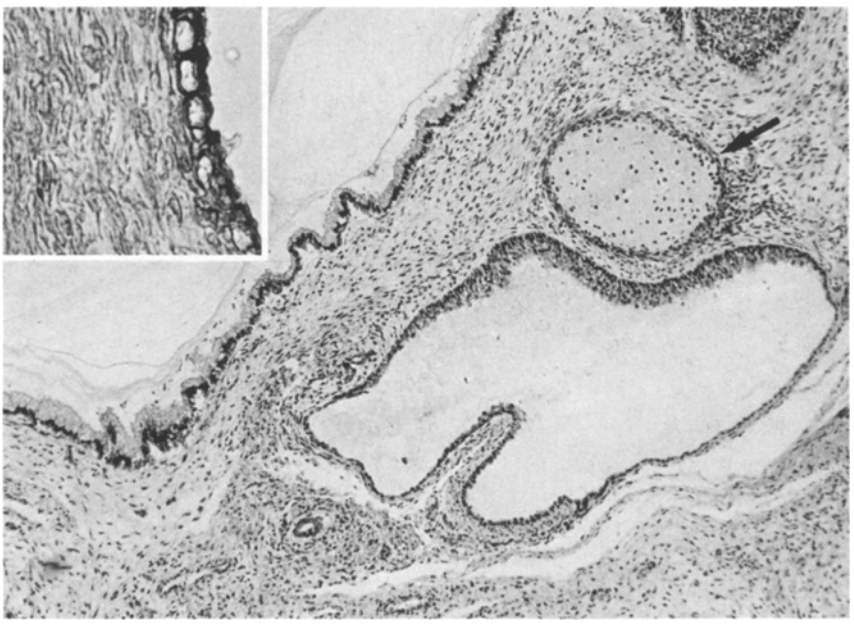

Fig. 7. Mediastinal adult teratoma. Mosaic of epithelial glandular and mesenchymal (cartilage: arrow) structures. Haematoxylin-Eosin. Mag. ×90 Inset: Keratin staining of glandular epithelia. Indirect immunoperoxidase method. No counterstaining. Mag. ×550

sheets. The normal cortico-medullary architecture of thymus epithelium was replaced by closely packed nearly solid epithelial complexes. Strongly labelled HC were scattered within these epithelial aggregates (Fig. 5B, C).

Tumours (Figs. 6A, B, 7). The present study included an investigation of 3 thymomas and 1 adult mediastinal teratoma. The classification of the thymomas is shown in Table 1. In both lympho-epithelial and pure epithelial thymomas, neoplastic epithelial cells reacted with 67K- and 55K-immune sera. The lymphoid tissue was completely negative (Fig. 6A, B).

The adult teratoma examined showed a mixture of non-labelled mesenchymal areas like primitive cartilage and positive epithelial glandular structures (Fig. 7).

Discussion

Within thymic epithelial cells, there are two immunologically different filament types, i.e. thin filaments and intermediate filaments of the keratin type (also described as keratin filaments or tonofilaments). Thin filaments (4–6 nm in diameter) were shown to be present in thymic epithelial and mesenchymal cells (Drenckhahn et al. 1979). Keratin filaments, however, were found to be a "marker" of epithelium-derived cells (Sun and Green 1978; Franke et al. 1978; Viac et al. 1980). In a previous study, Viac et al. (1980) showed that keratin antisera react specifically with epithelial cells of HC. Using these antisera, the

present investigation revealed that HC and the so-called epithelial reticulum of cortex and medulla have similar antigenic sites to keratinocytes of the stratum spinosum. Recent investigations on human epidermis and oral mucosa showed that the germinal compartment of dividing cells in the basal cell layer was not labelled by 67K-antibodies in contrast to the strongly stained differentiating compartment of suprabasal cells (Löning et al. 1980). For this reason, it may be assumed that a great part of normal cortical and medullary epithelial cells of the thymus are differentiated cells. Because keratinocytes of both HC and epithelial reticulum express 55K- and 67K-antigenic sites, it is suggested that there are no apparent qualitative differences in keratin polypeptide distribution between these keratinocyte populations, as in the epidermis, between basal and suprabasal cells (see Fuchs and Green 1980; Viac et al. 1980; Löning et al. 1980). The observation of secretory processes in keratinocytes of HC (Vetters and Macadam 1973) and epithelial reticulum (Clark 1966; 1968) may also be an indication of differentiation in both cell populations. Among those secretion products mucins (Henry 1966), secretory component (Tomasi and Yurchak 1972), thymosin (Mandi and Glant 1973) and FTS (Facteur Thymique Serique, Schmitt et al. 1980) were demonstrated histochemically or immunoenzymatically.

A further object of this study was the light microscopical analysis of epithelial arrangement in normal and altered thymuses. Although the epithelial framework of thymus is well known from electron microscopic studies (van Haelst 1967; Mandel 1968; Oláh et al. 1975) the availability of an epithelial cell "marker" may lead to a reinterpretation of previously described phenomena in the thymus. Among these phenomena there are the structure of HC (Kohnen and Weiss 1964; Bargmann 1967; Mandel 1968), the epithelial cortical borders around the lobules (Mandel 1968; Oláh et al. 1975) and the epithelial covering of blood vessels (Kostowiecki 1967; van Haelst 1967; Hwang et al. 1974; Bearman et al. 1975). There are many unanswered questions concerning the exact nature and function of HC (Kater and van Gorp 1969; Weiss 1977). We did not find any differences of keratin polypeptides distribution between outer and inner layers of HC. They are considered to originate from epithelial cells of the medulla (Mandel 1968, 1970) but from our point of view, both HC and most of medullary epithelial cells represent differentiated keratinizing cells. In HC, complete keratinization was described by electron microscopy (Kohnen and Weiss 1964; Mandel 1968). In thymic involution in particular, HC showed degenerative, cystic changes, whereas the epithelial reticulum appeared unaltered. Those alterations of HC are well known from conventional microscopical studies (Hammar 1926; van Haelst 1967; Rosai and Levine 1975). Interestingly, the central spaces of these HC were generally not labelled by keratin antisera, but were filled with an amorphous proteinaceous material, including nuclear dust and inflammatory cells. Granulocytes, which were plentiful, are believed to participate in degradation of keratinized HC (Oláh et al. 1975). On the other hand, Blau (1973) supposed that HC provide a site of thymocyte death. Our findings support the idea that most of the cellular material inside those cystic HC represents fragments of mesenchymal cells, in particular of granulocytes and lymphocytes. However, it may be that the cellular debris inside HC changes its antigenic properties and does not react with keratin antisera.

Epithelial cortical borders and the epithelial covering of blood vessels inside thymic lobules are also unique to this organ (van Haelst 1967; Bearman et al. 1975; Oláh et al. 1975). These structures are considered to constitute a barrier between blood and surrounding mesenchyme and the thymus parenchyma (see for review: Weiss 1977). In fact, 67K- and 55K-positive epithelial cells were demonstrated to surround the lobules and to enclose the interior blood vessels. However, these epithelial borders appeared to be rather irregular limitations. Raviola and Karnovsky (1972) have done tracer studies using peroxidase and other substances and have demonstrated that while the cortical vessels were impermeable, the barrier was imperfect in the region of the medullary venules where tracer substances escaped into the thymic parenchyma. From our static viewpoint, epithelial barriers in normal, involuted and hyperplastic thymuses seemed to be variable structures. Using keratin antisera, epithelial borders surrounding the cortex and epithelial sheaths enclosing vessels appeared often to be discontinous. We could not find differences between cortical and medullary vessels.

The variable arrangement of thymic epithelial cells was most striking in follicular hyperplasia. In this case, there was a marked change of thymic epithelial architecture (Rosai and Levine 1975). Almost solid epithelial complexes appeared which were located around germinal centers. These germinal centers were considered to be the sites of proliferation of abnormal clones of cells resistant to normal control processes of the thymic surveillance mechanisms (Burnet and Holmes 1964). The peculiar arrangement of epithelial cells in follicular hyperplasia was also observed in some biopsy cases (Löning et al. 1980). Further studies are needed to clarify the relevance of epithelial aggregates during thymus germinal center formation.

Our preliminary studies on thymic tumours (Rosai and Levine 1975; Otto and Hüsselmann 1978) showed that keratin antisera provide a light microscopical tool with which to recognize neoplastic epithelial cells. Mesenchymal areas as demonstrated in a mediastinal adult teratoma were negative. In future, the detection of keratin antigens may be of value in the sometimes difficult differential diagnosis of mediastinal tumours.

Acknowledgements. The authors wish to thank Miss C. Schürmann and Miss I. Brandt for skillfull technical assistance. We gratefully appreciate the kind gift of keratin antisera of Professor Dr. J. Thivolet and Madame M.J. Staquet.

References

Bargmann W (1967) Histologie und mikroskopische Anatomie des Menschen. 6. Auflage. Georg Thieme Verlag, Stuttgart, p 318–327

Bearman RM, Bensch KG, Levine GD (1975) The normal human thymic vasculature: an ultrastructural study. Anat Rec 183:485–498

Blau JN (1973) Hassall's corpuscles – A site of thymocyte death. Br J exp Path 54:634–637

Burnet FM, Holmes MC (1964) Thymic changes in the mouse strain NZB in relation to the auto-immune state. J Path Bact 88:229–241

Caselitz J, Löning T, Staquet MJ, Seifert G, Thivolet J (1981) Immunocytochemical demonstration of filamentous structures in the parotid gland. Occurrence of keratin and actin in normal

and tumoral parotid gland with special respect to the myoepithelial cells. J Cancer Res Clin Oncol (in press).
Clark SL (1966) Cytological evidence of secretion in the thymus. The thymus: experimental and clinical studies. Ciba Foundation Symposium. I and A Churchill, Ltd London, p 3
Clark SL (1968) Incorporation of sulfate by the mouse thymus: its relation to secretion by medullary epithelial cells and to thymic lymphopoiesis. J Exp Med 128:927–957
Drenckhahn D, von Gaudecker B, Müller-Hermelink HK, Unsicker K, Gröschel-Stewart U (1979) Myosin and actin containing cells in the human postnatal thymus. Ultrastructural and immunohistochemical findings in normal thymus and in myasthenia gravis. Virchows Arch B Cell Path 32:33–45
Franke WW, Weber K, Osborn M, Schmid E, Freudenstein C (1978) Antibody to prekeratin: decoration of tonofilament-like arrays in various cells of epithelial character. Exp Cell Res 116:429–445
Fuchs E, Green H (1980) Changes in keratin gene expression during terminal differentiation of the keratinocyte. Cell 19:1033–1042
Hammar IA (1926) Der Menschenthymus in Gesundheit und Krankheit. Z Mikrosk Anat Forsch 6:1–560
Henry K (1966) Mucin secretion and striated muscle in the human thymus. Lancet 22:183–185
Hwang WS, Ho TY, Luk SC, Simon GT (1974) Ultrastructure of the rat thymus. A transmission, scanning electron microscope and morphometric study. Lab Invest 31:473–487
Kater L, van Gorp HM (1969) Morphological aspects of Hassall's corpuscles. Path Europ 4:361–369
Kohnen P, Weiss L (1964) An electron microscopic study of thymic corpuscules in the guinea pig and the mouse. Anat Rec 148:29–57
Kostowiecki M (1967) Development of the so-called double-walled blood vessels of the thymus. Z Mikr Anat Forsch 77:401–431
Löning T, Burkhardt A, Seifert G, Gebbers JO (1977) Zur Typisierung der Immunglobuline in oralen Leukoplakien und Karzinomen. Dtsch Z Mund-Kiefer-Gesichts-Chir 1:168–177
Löning T, Staquet MJ, Thivolet J, Seifert G (1980) Keratin polypeptides distribution in normal and diseased human epidermis and oral mucosa. Immunohistochemical study on unaltered epithelium and inflammatory, premalignant and malignant lesions. Virchows Arch A Path Anat and Histol 388:273–288
Löning T, Viac J, Caselitz J, Schmitt D, Otto HF, Thivolet J (1980) Immunocytochemical and ultrastructural investigation of keratin and actin filaments in thymus tumours. (in prep.)
Mandel T (1968) The development and structure of Hassall's corpuscules in the guinea pig. A light and electron microscopic study. Z Zellforsch 89:180–192
Mandel T (1970) Differentiation of epithelial cells in the mouse thymus. Z Zellforsch 106:498–515
Mandi B, Glant T (1973) Thymosin-producing cells of the thymus. Nature (New Biol) 246:25
Oláh I, Röhlich P, Törö I (1975) Ultrastructure of lymphoid organs. An electron-microscopic atlas. Masson, Paris, p 105–147
Otto HF, Hüsselmann H (1978) Klinisch-pathologische Studie zur Klassifikation und Prognose von Thymustumoren. I. Histologische und ultrastruktur-pathologische Untersuchungen. Z Krebsforsch 91:81–102
Raviola E, Karnovsky MJ (1972) Evidence for a blood-thymus barrier using electron-opaque tracers. J Exp Med 136:466–498
Rosai J, Levine GD (1975) Tumors of the Thymus. Armed Forces Institute of Pathology, Washington, D.C.
Sainte-Marie G (1974) Tridimensional reconstruction of the rat thymus. Anat Rec 179:517–526
Schmitt D, Monier JC, Dardenne M, Pleau JM, Deschaux P, Bach JF (1980) Cytoplasmic localization of FTS (Facteur thymique serique) in thymic epithelial cells. An immunoelectronmicroscopical study. Thymus (in press)
Sun TT, Green H (1978) Immunofluorescent staining of keratin fibers in cultured cells. Cell 14:469–476
Tomasi TB, Yurchak AM (1972) The synthesis of secretory component by the human thymus. J Immunol 108:1132–1135
Van Haelst U (1967) Light and electron microscopic study of the normal and pathological thymus of the rat. I. The normal thymus. Z Zellforsch 77:534–553

Van Haelst U (1967) Light and electron microscopic study of the normal and pathological thymus of the rat. II. The acute thymic involution. Z Zellforsch 80:153–182

Vetters JM, Macadam RF (1973) Fine structural evidence for hormone secretion by the human thymus. J Clin Pathol 26:194–197

Viac J, Schmitt D, Staquet MJ, Thivolet J (1980) Epidermis-thymus antigenic relations with special reference to Hassall's corpuscules. Thymus 1:319

Viac J, Schmitt D, Staquet MJ, Thivolet J, Ortonne JP, Bustamante R (1980) Binding specifity of guinea pig anti-alpha keratin polypeptide sera on human keratinocytes: comparison of their receptors with those of human epidermal cytoplasmic antibodies. Acta Derm Venereol (Stockh) 60:189–196

Viac J, Staquet MJ, Goujon C, Thivolet J (1980) Experimental production of antibodies against stratum corneum keratin polypeptides. Arch Dermatol Res 267:179–188

Weiss L (1977) The thymus. In: Weiss L, Greep RO (eds) Histology, 4th ed. McGraw-Hill, p 503–522

Accepted January 19, 1981

Note Added in Proof

Recently, Von Gaudecker and Müller-Hermelink (Ontogeny and organization of the stationary non-lymphoid cells in the human thymus. Cell Tiss Res 207:287–306) investigated embryos (8th–16th gestational week) by conventional electron microscopy and found differences between cortical and medullary epithelial cells. At the present time, we have not analyzed the distribution of small and large keratins in thymic tissue of this age.

Our results of keratin staining in thymomas were recently confirmed by Battifora et al. (Battifora H, Sun TT, Bahu RM, Rao S (1980) The use of antikeratin antiserum as a diagnostic tool: thymoma versus lymphoma. Hum Pathol 11:635–641).