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Editors

Atlas of Thymic Pathology

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Preface

This book is a comprehensive, highly illustrated guide of thymic pathology for postgraduate residents, students, clinicians, and practicing pathologists. The book begins with reviewing the normal thymic structure and its immunohistochemical profile. The next section deals with the gross images of thymic lesions/tumors and histomorphology of nonneoplastic diseases, benign and malignant thymic tumors, with detailed descriptions of over 300 full-color photomicrographs accompanied by relevant clinical and radiology pictures and anatomical drawings. All photographs are complemented by short legends describing the illustration and providing relevant information. In addition to surgical pathology, a chapter is dedicated to the cytology of thymic lesions which is described rarely in available textbooks and literature. All types and stages of the most common thymic lesion that is thymoma is discussed in detail. A brief overview of the molecular pathology of thymic tumors is provided at the last along with the pathology of rare thymic lesions and lymphomas. The book is written by leading experts in thymic pathology, radiology, and surgical disciplines from the USA, Europe, Japan, and India. The book addresses the expanded role of pathologists in patient care of thymic lesions.

We hope this book will serve as a practical reference handbook of thymic pathology and help readers to understand and diagnose thymic lesions/tumors for correct patient management.

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Acknowledgments

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About the Editors

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Dr Jain has more than 260 peer-reviewed national and international publications to her credit. She authored a chapter in the 2015 WHO classification and is an editorial board member and author for the upcoming 2020 WHO classification of the lung, pleura, thymus, and heart. A member of the International Association for Study of Lung Cancer (IASLC) pathology committee and a section editor (Molecular Cytopathology) for the journal *Archives of Pathology and Laboratory Medicine*, Dr Jain has received many awards in recognition of her contributions.

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Dr Bishop is an expert in the surgical pathology diagnosis of head and neck, endocrine, and thoracic diseases. He has published more than 200 journal articles, 19 book chapters, and 7 books. Further, he contributed 15 chapters to the most recent WHO Classification of Head and Neck Tumors and is the lead author of the upcoming *AFIP Fascicle of Salivary Gland Tumors*. He is the editor-in-chief of *Seminars in Diagnostic Pathology*, an associate editor for *Modern Pathology* and *JAMA Otolaryngology—Head and Neck Surgery* and a member of the several additional editorial boards.

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The Normal Thymus

1

Alexander Marx

The thymus is a primary lympoid organ. It consists of epithelial cells, hematopoietic cells and mesenchymal cells and generates T cells from immature, bone marrow-derived precursors. Through selection processes, the T cells become functional and largely tolerant toward self-antigens and are of key importance for adaptive immune responses. Thymic failure, particularly if congenital, predisposes to life-threatening infections, neoplasia, and autoimmune diseases [1].

1.1 Embryology

The endoderm of the third pharyngeal pouches on both sides of the neck gives rise to "thymic epithelial cells" (TECs). From week 6 of gestation onward, the solid epithelial thymus *anlage* is present. By week 7, the common thymic/parathyroid primordia are established. The thymic components of the primordia descent along the carotid artery and behind the lower pole of the thyroid to the pre-cardiac region where they fuse [2].

From week 8 onward, the differentiation of cortical and medullary TECs (mTECs, cTECs) begins. By week 16, cortical and medullary compartments are established.

The developmentally indispensable thymic capsule and septae originate from neural crest-derived mesenchymal cells from week 7 onward [3, 4]. The earliest T cell precursors are present at week 8 [2]. T cell maturation and the generation of three-dimensional thymic lobes depend on interactions between NOTCH1 and DLL4 on T cells and TECs, respectively. Early Hassall corpuscles can be recognized by week 12 [5]. Mature T cells leave the thymus between week 14 and 16. The transcription factor FOXN1 is indispensable for the development of the thymus throughout embryonal and adult life [2]. Its defective expression elicits the nude phenotype and immunodeficiency in mice and humans [6, 7].

1.2 Normal and Ectopic Location of the Thymus

The normal position of the thymus is the anterosuperior mediastinum between the upper end of the sternum, the level of the fourth costal cartilage, the upper part of the pericardium and the pre-tracheal fascia, and the dorsal plain of the upper part of the sternum, costal cartilages, and intercostal muscles [8]. The lateral boundaries may extend beyond the phrenic nerves. Ectopic extensions comprise the cervical region up to the base of the skull, the mandibles and salivary glands, the middle and posterior mediastinum, and the intrapericardial and pleural spaces. The frequency of ectopic thoracic and cervical thymic tissue depends on the type of workup. On the microscopic level, frequencies amount to 20–50% [9, 10] but only to 1% in routine autopsies [11].

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Fig. 1.1 Juvenile thymus following the removal of mediastinal fat with conspicuous upper and lower horns

1.3 Macroscopy

The thymus is composed of two lobes. Their fibrous capsules stick together in the midline. Two upper and two lower "horns" can usually be recognized (Fig. 1.1). In children and adolescents, the cut section of the thymus resembles the cut surface of a lymph node. During the course of involution (see below) it becomes more and more yellow and is barely detectable in the elderly. The average thymus weight is about 15 g at birth (range 5–25 g), reaches a maximum of around 40 g (20–50 g) at 10–15 years of age, and declines thereafter, reaching 10–15 g (range 5–30 g) by age 60 [12].

1.4 Histology

Thymic lobes are composed of many lobules. During childhood, each lobule shows a central medullary compartment that is completely surrounded by an outer cortical layer. From adolescence onward, the architecture gets progressively disturbed, and medullary areas more and more abut on mediastinal fat (Fig. 1.2). In a strict sense, the third thymic compartment, the perivascular space (PVS), is an extrathymic space between the continuous basal membrane of the outermost TECs of thymic lobes and the basal membrane of the vessels that enter and leave the thymus along the septae (Fig. 1.3). Hematopoietic cells that enter or leave the thymus must cross the PVS to egress from or enter into blood vessels, respectively [13]. In the thymic capsule, interlobular septae, and medulla, efferent lymphatic vessels can be found [14].

Epithelial Cells Cortical and medullary TECs (cTECs and mTECs) show different histological features: The stellate-shaped cTECs are quite easily detectable due to their large, round nuclei with conspicuous nucleoli, while mTECs are hard to identify among the lymphocytes due to their small, oval nuclei with inconspicuous nucleoli (Fig. 1.4a–d). The distinction of cTECs from mTECs can be achieved by immunohistochemistry, using antibodies, e.g., to the cortex-specific proteasome subunit, Beta5t, and mTEC-restricted proteins such as CD40, Claudin-4, and the tolerance-inducing autoimmune regulator, AIRE [15–17] (Table 1.1; Fig. 1.5). AIRE-positive mTECs can develop toward Hassall corpuscles (HC) on downregulation of AIRE. HC are onion-shaped accumulations of concentrically arranged squamoid epithelial cells that can show keratohyalin granules, lose their nuclei toward their cornified centers, and may become calcified or cystic (Fig. 1.6). In contrast to other TECs, HC express cytokeratin 10 and involucrin and fail to express HLA-DR and DP [18]. Since T cell maturation is necessary for HC development, they are lacking in thymuses from patients with T cell developmental defects (historically called "thymic dysplasia"). During aging the number of HC declines [19]. HC have immune tolerogenic functions through shedding of autoantigens and their impact on the development of regulatory T cells [20].
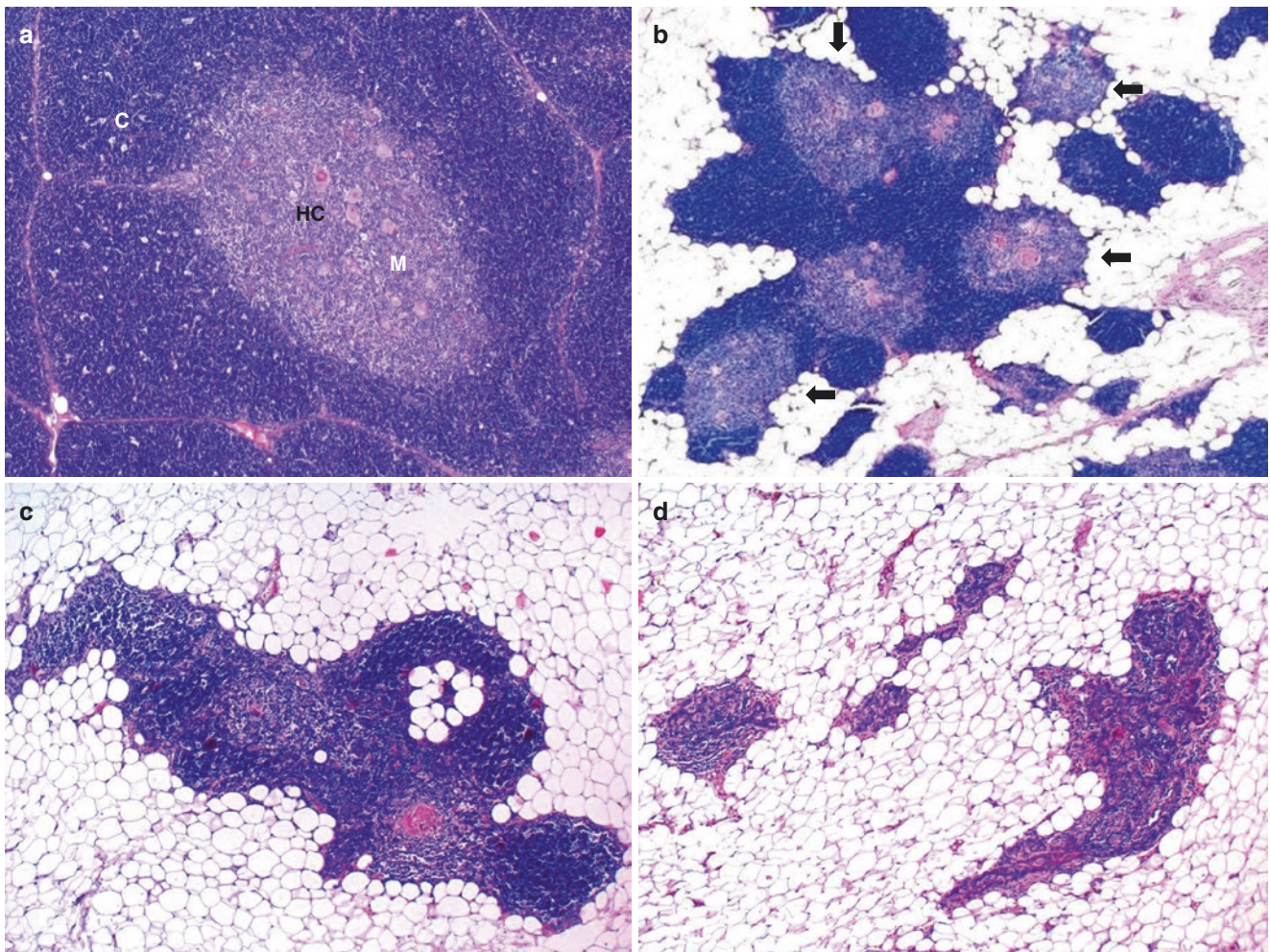
Fig. 1.2 Histology of thymuses in relation to age. (**a**) Thymus of a 1-year-old child: distinct lobular architecture with well-developed cortical areas (C) that completely envelope a medullary region (M); many small Hassall corpuscles (HC) and absence of interlobular fat are typical. (**b**) Thymus of a 30-year-old adult with increase of interlobular fat and medullary areas directly abutting on adipocytes (arrows). (**c**) Thymus of a 50-year-old adult with further loss of lymphoepithelial parenchyma and more severe distortion of the cortico-medullary architecture. (**d**) Thymus of a 70-year-old adult with near defect of cortical areas and paucity of lymphocytes within epithelial strands (HE, **a**–**d**)
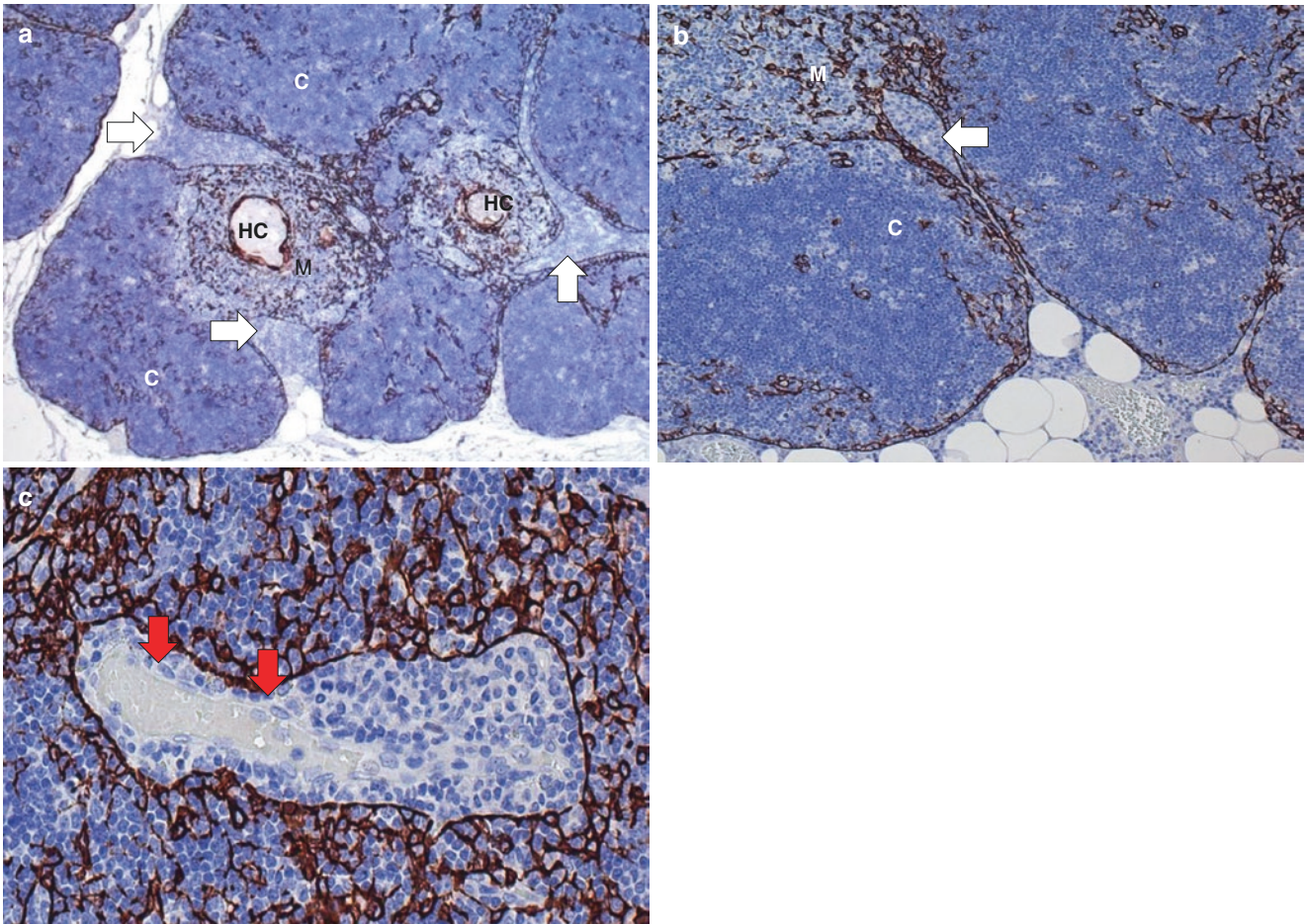
Fig. 1.3 Perivascular spaces (PVS) as highlighted by keratin 19 immunohistochemistry in a normal thymus. (**a**) Light-staining PVS are epithelial-free spaces reaching from the perithymic fat and along the septae to the cortico-medullary junction (white arrows); the PVS are filled with lymphocytes, the majority of which are mature T cells (C, cortex; M, medulla; HC, Hassall corpuscle). (**b**) Sharp delineation between an epithelial-free PVS (arrow) and cortex (C) and medulla (M) through a continuous layer of thymic epithelial cells; the disruption of the layer around PVSs is a typical sequela of lymphofollicular hyperplasia in myasthenia gravis. (**c**) Small capillary vessel (red arrows) within a PVS (immunoperoxidase, Keratin 19, **a**–**c**)
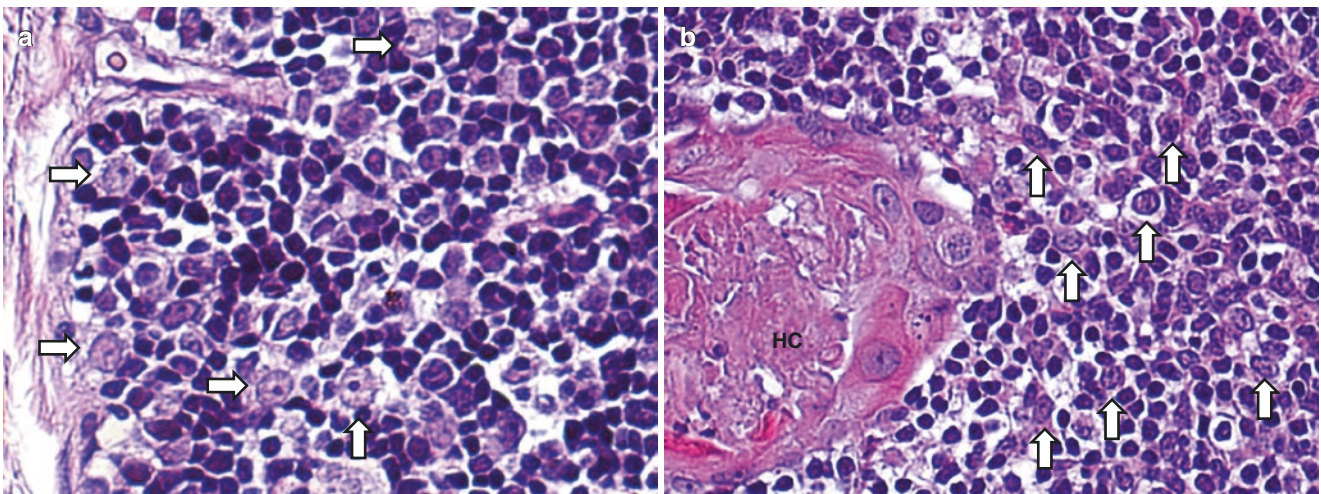
Fig. 1.4 Cytological features of normal cortical and medullary thymic epithelial cells (cTEC, mTECs). (**a**) cTECs with medium-sized to large vesicular nuclei with conspicuous nucleoli (arrows). (**b**) Barely visible mTECs (arrows) outside a Hassall corpuscle (HC) with small- to medium-sized nuclei and inconspicuous nucleoli. (**c**) Nuclei of cTEC highlighted by P40 staining. (**d**) Significantly smaller nuclei of mTEC outside HC highlighted by P40 immunohistochemistry (HE, **a**, **b**; immunoperoxidase, **c**, **d**)
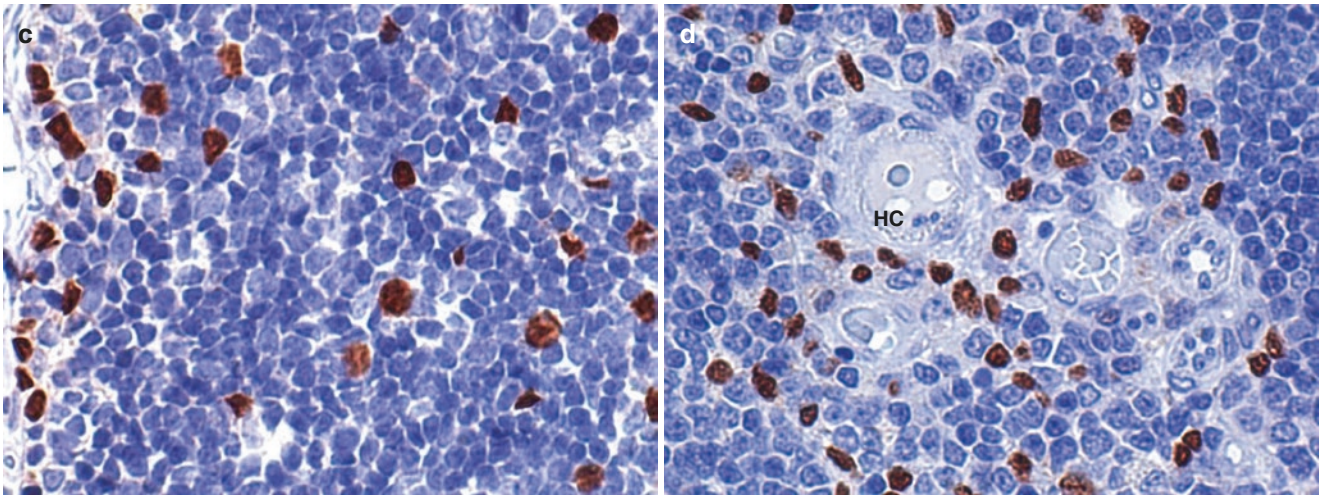
Fig. 1.4 (continued)

Table 1.1 Markers differentially expressed in the thymic cortex and medulla [31]

Cortical markers	Beta5t
	PRSS16
	Cathepsin V
	TdT*
Medullary markers	CK10
	CD40
	Claudin-4
	AIRE
	Involucrin
	Desmin, titin, myogenin**
	CD20, CD23***

*on immature T cells, **in myoid cells, ***on thymic B cells
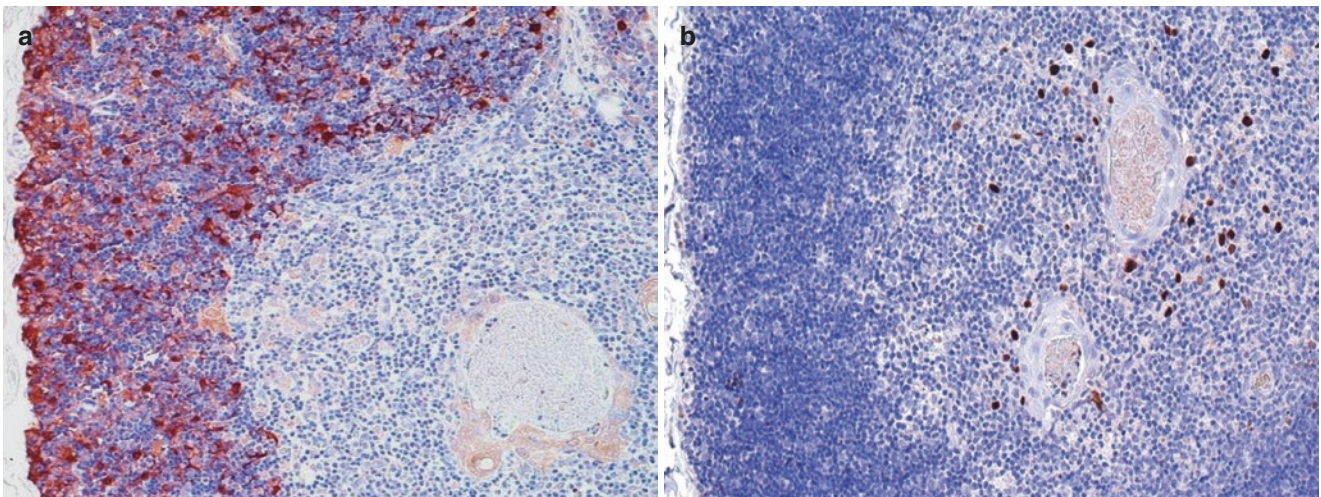
Fig. 1.5 Compartment-specific and largely unspecific epithelial markers in the normal thymus. (**a**) Expression of the thymus-specific proteasome subunit Beta5t exclusively in thymic epithelial cells of the cortex (C). HC, Hassall corpuscle. (**b**) Nuclear expression of the autoimmune regulator (AIRE) exclusively in a subset of medullary thymic epithelial cells. (**c**) Expression of keratin 19 in virtually all cortical and medullary epithelial cells and epithelial cells surrounding perivascular spaces (*). (**d**) P40 expression in the nuclei of almost all cortical and medullary epithelial cells (immunoperoxidase, **a–d**)
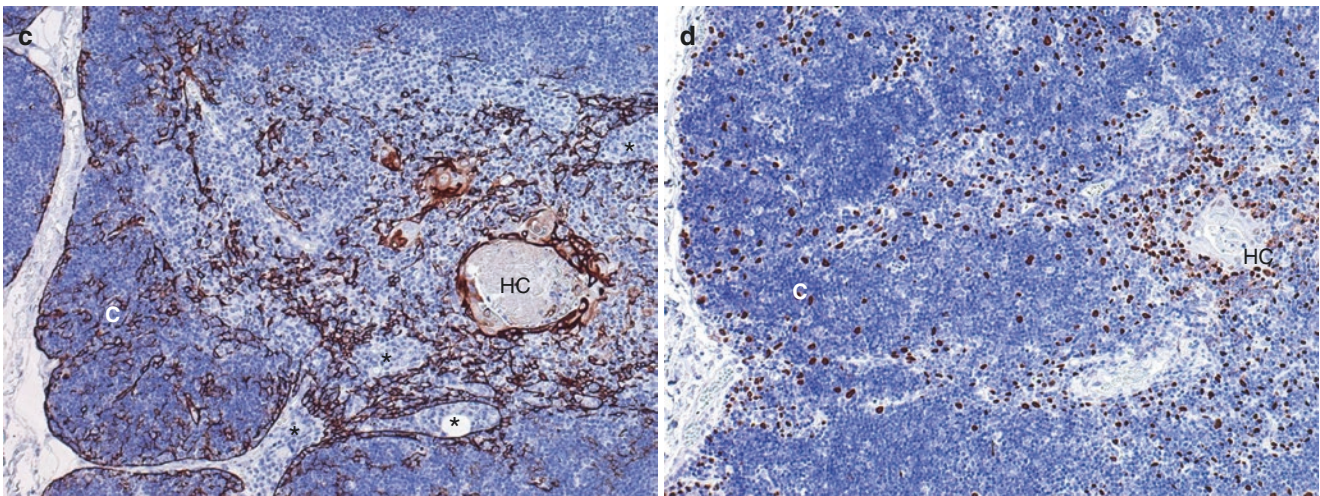
Fig. 1.5 (continued)
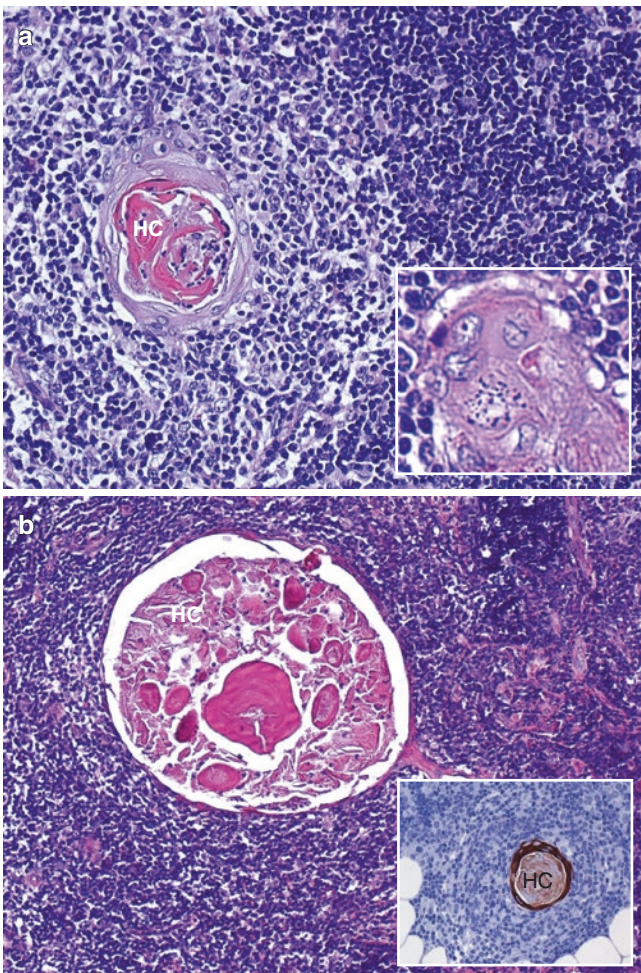
Fig. 1.6 Hassall corpuscle (HC) (**a**) HC with regressive changes and apoptotic cells in the medulla; inset, HC composed of vital, epidermoid cells with blue keratohyalin bodies; (**b**) HC with cystic enlargement containing debris; inset, typical expression of keratin 10 in the outer epithelial layers of a HC (HE, **a**, **b**; immunoperoxidase, keratin 10, inset)

T Cells (Thymocytes) On immunohistochemistry, the cortex appears completely occupied by immature TdT+ thymocytes almost all of which co-express CD1a, CD99, CD3, CD4, CD5, and CD8 and are negative for CD10 and CD34 (so-called CD4/CD8 double-positive (DP) thymocytes) with a Ki67 index >90% (Fig. 1.7a, b). By contrast, the minor, CD34+ subset, CD10+ CD1a− subcapsular subset, and the CD4+CD8−CD3− immature single-positive (iSP) thymocyte subset can only be detected by flow cytometry [21]. In the medulla, almost all cells show a TdT-negative phenotype and a low Ki67 index and belong either to the CD3+CD4+CD8− or CD3+CD4−CD8+ so-called "single-positive" (SP) T cell subsets. Cortical and medullary thymocytes share expression of CD3 and CD5, with particularly strong CD5 expression in the medulla (Fig. 1.7c, d).

B cells normally occur only in the medulla. The majority is round, while a minority is "asteroid shaped" (i.e., dendritic) if stained for CD20 [22]. The B cell content of medullary areas is highly variable, but B cells are always present. The "asteroid subset" shows a characteristic CD20+CD23+ CD21− profile (Fig. 1.8). Thymic B cells can originate through immigration from extrathymic mature B cell sources (e.g., lymph nodes) or through intrathymic development from immature precursors [23, 24]. Thymic B cells are HLA-DR+ and involved in T cell tolerance through negative T cell selection and the induction of regulatory T cells [24, 25].

Macrophages and Dendritic Cells Macrophages occur in the cortex and medulla, while dendritic cells (DCs) are largely restricted to the medulla with a major focus on the cortico-medullary junction. Macrophages are important for the removal of dying thymocytes during T cell selection [18, 26] and are CD68+ and/or CD163+ (Fig. 1.9a). The small and round subset is strongly HLA-DR+, while the large,
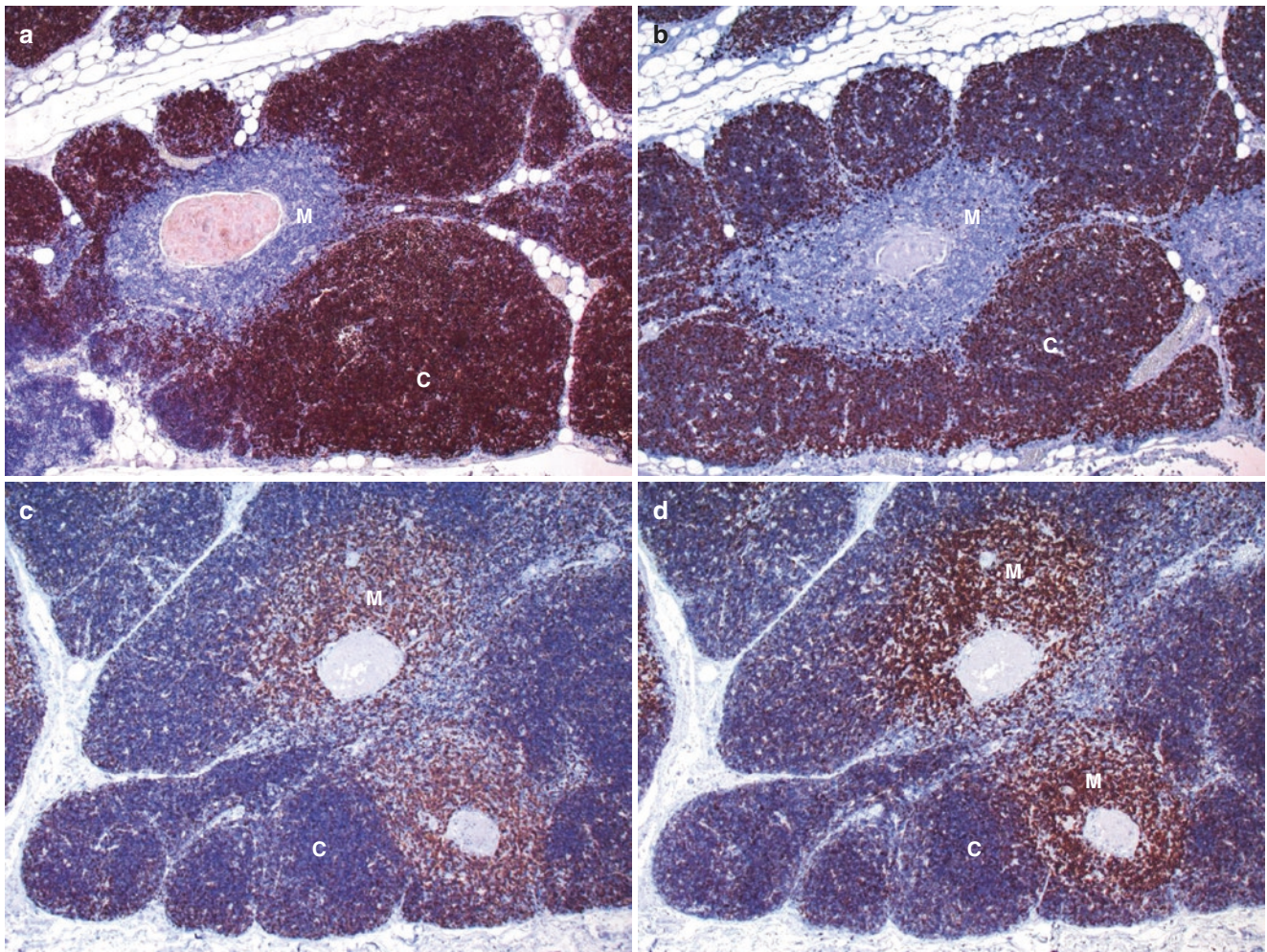
Fig. 1.7 T cells in the normal thymus. (**a**) Restriction of immature, TdT-positive T cells to the cortex (C) with labelling of virtually all lymphoid cells. (**b**) High Ki67 index (>90%) of cortical thymocytes as compared to few Ki67-positive cells in the medulla (M). (**c**) CD3 expression on immature and mature T cells in both compartments. (**d**) Particularly strong expression of CD5 on T cells in the medulla (M) (immunoperoxidase, **a**–**d**)
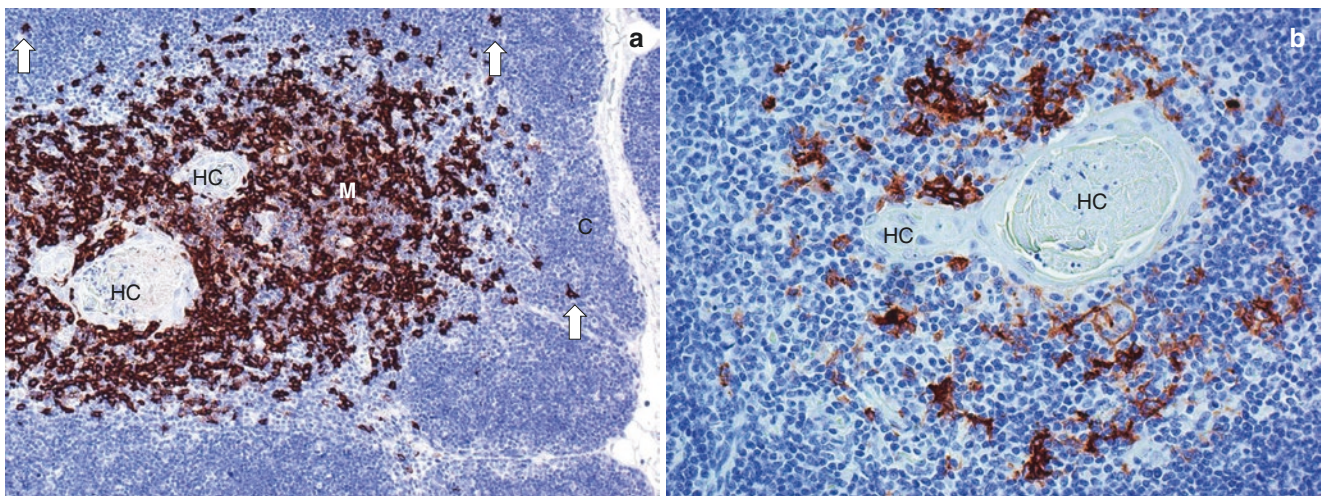
Fig. 1.8 B cells in the normal thymus. (**a**) Mainly round CD20+ B cells in the thymic medulla (M) with apparent "spillover" of single B cells into the cortex (arrows). (**b**) The "asteroid" B cell subset that is generally blurred by the overwhelming majority of round B cells on CD20 immunohistochemistry can be highlighted by CD23 stains; CD23+ B cells must be distinguished from CD23+ follicular dendritic cells that form networks in thymic follicular hyperplasia (immunoperoxidase, **a**, **b**)

Fig. 1.10 Thymic myoid cells (TMCs) in the normal thymus: occurrence of desmin-positive TMCs exclusively in the medulla (here abutting on fat cells, right upper part); round, rhabdomyoblast-like TMCs (white arrows) and more elongated, myotube-like TMCs (black arrows) with vague cross-striations are present in the vicinity of a Hassall corpuscle (HC) (immunoperoxidase, desmin)

muscle-type nicotinic acetylcholine receptors that likely play a role in the pathogenesis of myasthenia gravis [32, 33]. The normal function of TMCs is unclear, but it has been speculated that they release autoantigens and, thereby, endow DCs with the potential to induce muscle-specific T cell tolerance through negative selection [34].

Fig. 1.9 Macrophages and dendritic cells in the normal thymus. (**a**) Occurrence of CD68-positive macrophages throughout the thymus, including a Hassall corpuscle (HC); greatest frequency in the cortex (C); CD163-positive macrophages show a similar distribution and abundance (not shown). (**b**) CD11c-positive dendritic cells are largely restricted to the medulla, including the cortico-medullary junction; minor "spillover" CD11c-positive cells to the cortex (C)

stellate-shaped "starry sky macrophages" mainly of the cortex may contain apoptotic thymocytes and are HLA-DR^{low} [27]. DCs can a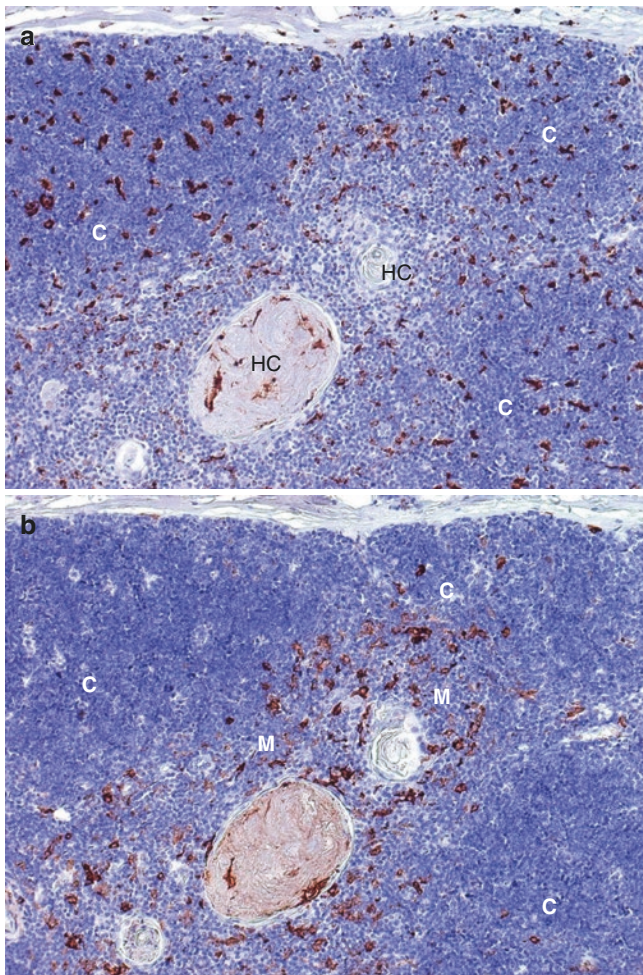rise from intrathymic precursors or enter the thymus as mature DCs from outside [28]. They are strongly HLA-DR+ and promote negative T cell selection and the induction of regulatory T cells [20]. Conventional DCs express CD11c (Fig. 1.9b) and may be AIRE+ [29], while the rare plasmacytoid DCs express CD123.

Myoid Cells Thymic myoid cells (TMCs) are fetal-type striated muscle cells of unknown origin in the medulla [30]. They express contractile proteins, including titin [31]. When stained for desmin, they resemble round, immature myoblasts or elongated myotubes (Fig. 1.10). Because they are non-innervated cells, TMCs express fetal and adult skeletal

1.5   Thymic Function

The thymus has three key functions: i) to recruit hematopoietic precursor cells from the blood into the thymus and drive their multistep maturation and expansion (Fig. 1.11) [21, 35], leading to a diverse repertoire of α/βT cells that can recognize millions of antigenic peptides if they are presented by antigen-presenting cells (APCs) on class I and II major histocompatibility (MHC) molecules ("positive selection"); ii) to eliminate from the functional T cells the subset of autoreactive T cells ("negative selection") through the action of mTECs, DCs, and thymic B cells mainly in the medulla [29, 36, 37]; and iii) to generate immunosuppressive CD4+CD25+ FOXP3+ regulatory T cells (Tregs) that are indispensable for keeping autoreactive T cells in check that inevitably escape from negative selection and reach the peripheral immune system [38]. An essential factor for negative selection is the transcriptional "autoimmune regulator," AIRE, that drives the expression of thousands of self-antigens in a subset of mTECs and endows them with the capacity to kill T cells if they show high affinity for MHC-presented self-peptides [39]. The thy-

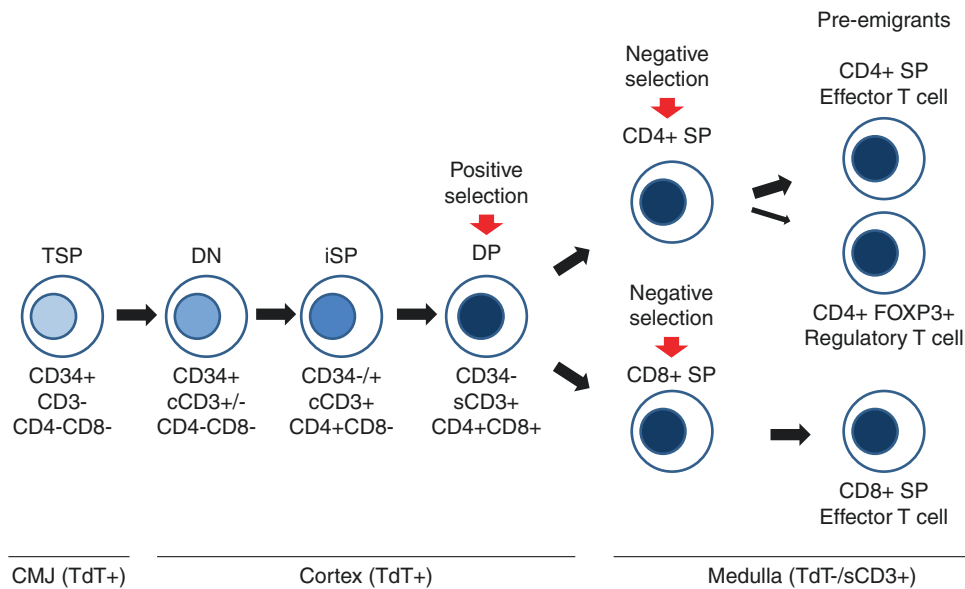**Fig. 1.11** Maturation of alpha/beta T cells and main levels of operation of positive and negative T cell selection in the human thymus: *TSP* thymic-seeding precursors (the most immature T cell precursors entering the thymus), *DN* double-negative cells (in terms of CD4 and CD8 expression, *cCD3* denotes CD3 expression in the cytoplasm), *iSP* immature single-positive cells, *DP* double-positive cells (constitute more than 90% of the cortical thymocytes; *sCD3* denotes CD3 expression on the cell surface), *SP* single-positive T cells, and *pre-emigrants* the most mature T cells generated in the thymus that are ready to egress from the thymus at the cortico-medullary junction (CMJ) into perivascular spaces (PVS) and from there into PVS-borne blood vessels and the circulation

mus is also important for the generation of γ/δ T cells [40] and NKT cells [41].

amounts of IFN-γ increase the risk for inflammatory tissue reactions and autoimmunity [46].

1.6 Thymic Involution

Thymic involution denotes the physiological, age-related, and gradual replacement of functional thymic tissue by fat (see above Figs. 1.1–1.3). Morphometry showed that involution starts in the first year of life and continues thereafter, leaving about 5% of thymic parenchyma by the age of 60 [19] . In a broader sense, involution includes thymic atrophy ("accidental involution") that happens through various "stressors" such as pregnancy, infection/inflammation, malnutrition, and cancer. Factors involved in thymic atrophy are corticosteroids, sex hormones, IFN-α, adipocyte-derived factors (e.g., LIF), TNF-α, IL6, and growth factors [42, 43]. Mechanisms that are operative in relation to age are declining levels of FOXN1, decreasing proliferative activity of TECs with age, exhaustion of TEC progenitor cells, and the declining capacity of cTECs to induce T lineage commitment through NOTCH1 signaling and of mTECs to induce tolerance through expression of self-antigen [44].

These age-related changes lead to a gradual accumulation of senescent T cells and—most likely—to an increased risk of infections and cancer with increasing age [45]. On the other hand, the relative resistance of senescent T cells to regulatory signals and propensity to generate increased

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Immunohistochemistry of Normal Thymus

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2.1 Introduction

Immunohistochemistry contributed, among other specialized techniques, to characterize the two main compartments of the human thymus, cortex and medulla, and their constituent cells.

Several antibodies have been raised which provide insight into the normal thymus as well as in the tumors derived from epithelial cells (ECs) in thymus, the thymoma and thymic carcinoma, collectively called thymic epithelial tumors (TETs) and variety of lymphoproliferative diseases occurring in the thymus. Table 2.1 shows a list of the immunohistochemical markers useful in the thymic microenvironment characterization by cell type: some are differentiation and/or cell lineage markers whereas others are functional biomarkers. Ki67 is a general proliferation marker [1–19]. Compartment-specific antibodies have also been raised, showing phenotypic differences between cortical epithelial cells (cEC) and medullary epithelial cells (mEC). A list of compartment-specific and of embryological regulatory markers is provided (Table 2.2) to the readers interested in specialized morphofunctional investigations [20–29].

2.2 Markers of the Epithelial Component

2.2.1 Cytoplasmic Markers

2.2.1.1 Cytokeratins

Cytokeratins (CKs) are cytoplasmic intermediate-sized filaments (tonofilaments) expressed by epithelia. The complex pattern of thymic CK staining includes almost all the CKs [1, 3, 30, 31]. The normal thymic EC of all compartments is CK19 positive; CK20, CK7, and CK14 are expressed by the subcapsular EC, mEC, and Hassall corpuscle (HC) EC, but not the cEC. Only HC expresses consistently CK13 and CK10. CK8 and CK18 are expressed by mEC and HC [1]. The CK pattern complexity reflects the morphological and functional heterogeneity of EC in the thymus [32] (Fig. 2.1).

2.2.2 Nuclear Markers

2.2.2.1 p63 and p40

p63 is a member of the p53 tumor suppressor gene family with structural homology to p53. In the human thymus of different ages, it was found that all thymic epithelial components, including subcapsular, cortical, and medullary ECs, express the nuclear p63 and its DN-p63α isoform, also called p40, with lesser intensity in HC [4]. In Fig. 2.2a the nuclei of all EC react with anti-p63 antibody, both in the cortex and in the medulla; in Fig. 2.2b the p63-positive subcapsular EC layer is better seen. A similar pattern is seen by staining with anti-p40, which highlights the nuclei of all EC types (Fig. 2.2c); in the medulla, only few ECs in the external part of HC are stained (Fig. 2.2d).

2.2.2.2 PAX8

PAX8 is a member of the paired box (*PAX*) family of transcription/developmental genes. PAX8 is a tissue-specific transcription factor involved in the embryonic development of the kidney, Müllerian organs, and thyroid. Recent studies

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Table 2.1 Routine markers for thymus characterization by immunohistochemistry

Marker	Notes on specificity/characterization/expressing cells	References
CK19	Cortical and medullary EC	[1]
CK AE1/AE3	Pankeratin marker (acidic and basic) of EC	[2]
CK 5/6	Non-keratinizing epithelia; basal-type CK	[3]
P63	Nuclear marker of all thymic EC	[4]
P40	It represents a DN-p63α isoform; nuclear marker of all thymic EC	[5]
PAX8 (paired box (*PAX*) family of transcription/ developmental genes	Transcription factor during embryonic development; expressed weakly in thymus	[6]
Glut1	Member of the mammalian facilitative glucose transporter family of passive carriers	[7]
CD1a	Immature cortical thymocytes and DC in thymic medulla	[8]
CD3	T cell lineage differentiation antigen	[9]
CD5	Non-lineage specific pan-T cell antigen; aberrantly expressed in some thymic carcinomas	[10]
CD4	T helper cells	[11]
CD8	T cytotoxic cells	[12]
CD10	Common acute lymphoblastic leukemia antigen (CALLA)	[13]
Terminal deoxynucleotidyl transferase (TdT)	Non-lineage specific immature T cell nuclear marker/reacting with T-Lb	[14]
LIM only 2 domain (rhombotin-like 1) (LMO2)	Negative in normal lymphoblasts, positive in T-Lb	[15]
CD20	B cell lineage marker	[16]
CD23	B cell lineage marker and follicular DC	[17]
Desmin	Myoid cells in thymic medulla	[18]
Ki67	All proliferating cells	[19]

EC epithelial cells, *cEC* cortical epithelial cells, *mEC* medullary epithelial cells, *CK* cytokeratin, *CD* cluster of differentiation, *DC* dendritic cells, *DN* Delta N, *T-Lb* T-lymphoblastic lymphoma

Table 2.2 Developmental and compartment-specific markers

Marker	Notes on role/characterization/expressing cells	References
FoxN1	cEC differentiation regulator	[20]
CD205	cEC and DC subset marker	[21]
Notch1	Transmembrane receptor regulating normal T cell development; expressed in T-ALL, not in normal T lymphoblasts	[22]
β5t proteasome	cEC	[23]
CD40	mEC	[24]
CK10	Terminally differentiated mEC	[25]
Claudins (Cld)	Membrane proteins involved in junctional complexes; mEC in thymus	[26]

cEC cortical epithelial cells, *mEC* medullary epithelial cells, *CK* cytokeratin, *CD* cluster of differentiation, *DC* dendritic cells, *T-ALL* T cell acute lymphoblastic leukemia

have shown that, among tumors, PAX8 is commonly expressed in epithelial tumors of the thyroid and parathyroid glands, kidney, thymus, and female genital tract [33].

The relevance of this marker in normal thymus is still to be established, whereas the anti-PAX8 antibody appears to be useful in the differential diagnosis of thymic carcinoma versus poorly differentiated lung carcinoma [34]. In the normal thymus, PAX8 (if using polyclonal antibodies) stains scattered subcapsular EC in the thymic cortex (Fig. 2.2e) and scattered EC in the thymic medulla, whereas ECs of HC are weak positive or mostly negative (Fig. 2.2f).

2.2.3 Other Markers

2.2.3.1 Glut-1

Glut-1 is a member of the mammalian facilitative glucose transporter family of passive carriers that function as an energy independent system for glucose transport down a concentration gradient [35]. In TET, Glut-1 expression was found to be dependent on histological subtypes [36]. The Glut-1 stain should be considered in the differential diagnosis between B3 thymoma and thymic carcinoma [37]. In the normal thymus Glut-1 is not expected to stain ECs significantly. However, in the examples shown here, faintly stained EC cytoplasm in clusters at the border of a peritumoral thymus or in the medulla itself (Fig. 2.3a) or at the border of a thymic cyst (Fig. 2.3b) are seen. This finding is of unknown significance.
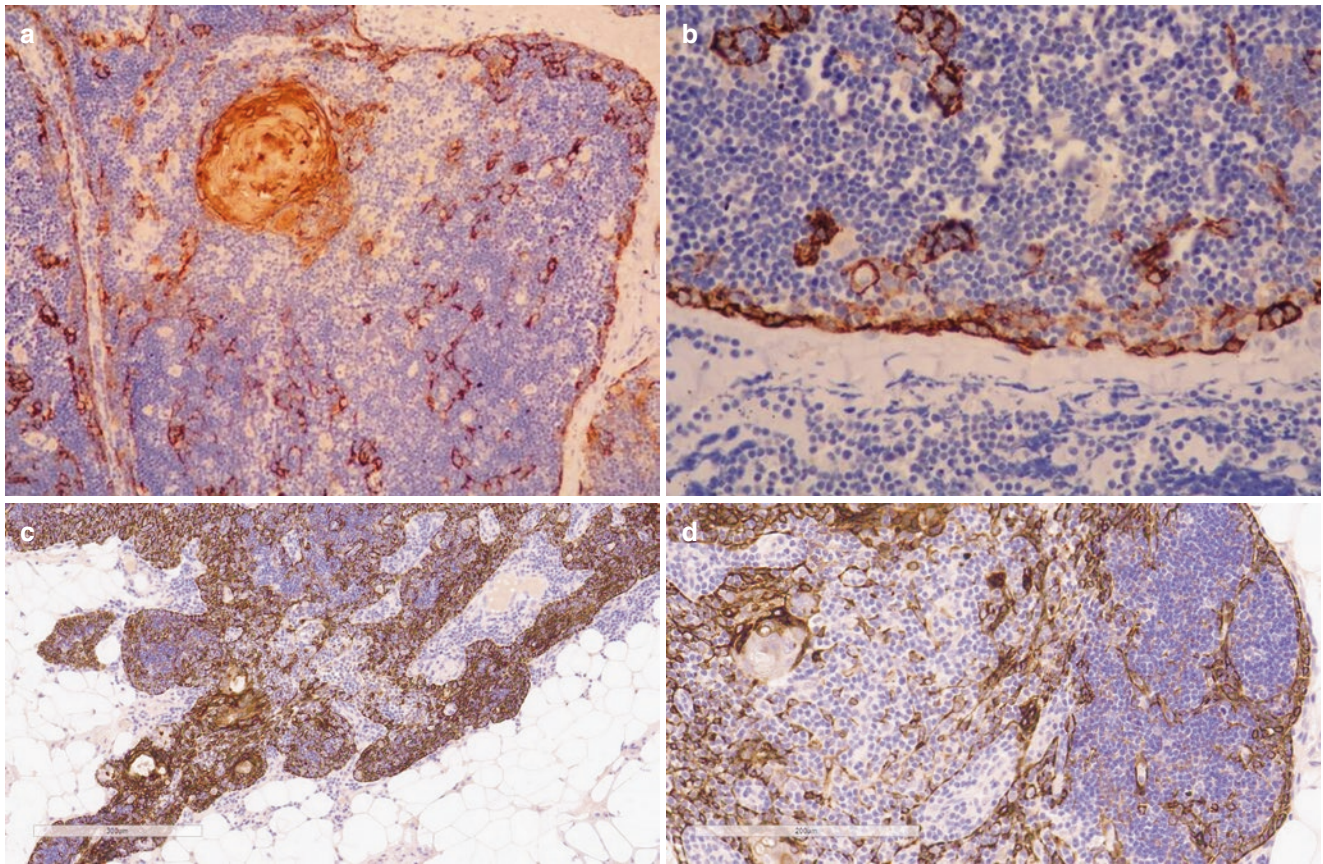
Fig. 2.1 Cytokeratin staining of normal thymus. A low magnification view of normal thymus stained with the anticytokeratin AE1/AE3 showing all the ECs, at the cortical border as well as into the cortex and in the medulla; a prominent HC is seen, mainly reacting at its periphery (**a**, 100×); at higher magnification (**b**, 200×) the subcapsular ECs are highlighted by the stain; a network of positive EC is seen in the cortex. The anticytokeratin antibody 5/6, marker of a basal layer type of CK, stains a very rich EC network (**c**, 100×); CK5/6 stains the cortical lobules as well as the mEC, both as a network and as single EC (**d**, 200×)
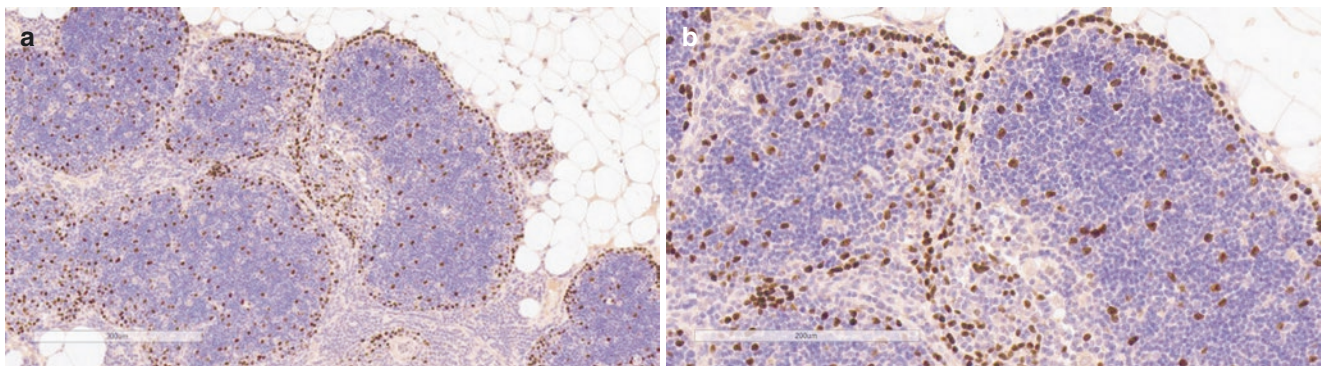

Fig. 2.2 p63, p40, and PAX8: (**a**, 100×), the nuclei of all EC react with anti-p63 antibody, both in C and in M; in (**b**, 200×), the p63-positive subcapsular EC layer is better seen. (**c**, 50×), A similar pattern is seen by staining with anti-p40, which highlights the nuclei of all EC types (**d**, 200×); in M, only few ECs in the external part of HC are stained. (**e**, 200×), in the normal thymus Pax 8 stained scattered subcapsular EC in the thymic C and scattered EC in the thymic M, whereas (**f**, 200x) ECs of HC are weak positive. *C* cortex, *M* medulla, *EC* epithelial cells, *HC* Hassall corpuscles