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Thymic Microenvironment at the Light Microscopic Level

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ABSTRACT The thymus is a primary lymphoid organ that serves the immune system by providing an optimal microenvironment for developing T cells to rearrange the genes encoding the T-cell receptor and to undergo positive and negative selection in shaping the peripheral T-cell repertoire. The microenvironment of the organ is peculiar among lymphoid organs, as the supporting stroma consists of reticular epithelial cells. Bone marrow-derived interdigitating cells and macrophages are the main accessory cell populations. The epithelium, interdigitating cells, and macrophages each contribute to the T-cell selection process. During the last decade knowledge has been gathered that these cell populations show a considerable heterogeneity, as documented for subcellular features and immunologic phenotype. This heterogeneity may reflect various stages in differentiation, but may otherwise be linked to the functional activity of the cells. The authors survey the major cell populations, i.e., epithelial cells and lymphocytes. Macrophages and interdigitating cells are briefly discussed. Emphasis is given to functional aspects of histologic/cytologic features. *Microsc. Res. Tech. 38:216–226, 1997.*

INTRODUCTION

In the histologic characterization of the thymus, and the interpretation of histologic observation, it is emphasised that the thymus is a very dynamic organ that rapidly changes under exogenous influence and involutes with age (Kendall, 1991; Kuper et al., 1992; Steinmann, 1985). The histologic picture presents only a momentary view of such processes. Most descriptions concern the fully developed thymus as most active during the first life period till sexual maturity (Boyd et al., 1993; Brekelmans and Van Ewijk, 1990; Kendall, 1991; Schuurman et al., 1993; Von Gaudecker, 1991). We have elsewhere reviewed the histology of the thymus in pathologic conditions (Huber et al., 1992; Schuurman and Kuper, 1995; Schuurman et al., 1991, 1992). Kendall (1995) has recently reviewed hemopoiesis in the thymus.

Individual lobules are variable in shape, size, and orientation. Each lobule contains a central part, the medulla, and a peripheral part, the cortex (Fig. 1). These are easily distinguished by the difference in lymphocyte density, which is higher in the cortex. The cortex is further differentiated into a subcapsular area, the outer cortex and the inner cortex. Between cortex and medulla, the cortico-medullary region is recognized as an area rich in blood vessels, where septa can reach the medulla. The medulla can form small buds that reach deep into the cortex and sometimes come close to the capsule; also cortex-like areas can cross the medulla (Sainte Marie, 1974). From the capsule and septa, trabeculae spread through the cortex to the medulla.

The histology of the thymus is comparable in all vertebrates, e.g., a lobulated organ with corticomedullary organization. Its association with the pharyngeal epithelium is especially prominent in some fish species (e.g., bony fish, teleost), in which the organ lies close to the gill epithelium (Manning, 1981; Wester et al., 1994; Zapata and Cooper, 1990).

In the following sections, we describe the supporting stroma of epithelial cells, accessory cells from bone marrow origin, i.e., macrophages and interdigitating cells, and finally the lymphoid component. Figure 2 presents some examples of the identification of (subsets of) these cells.

THYMIC STROMA AND EPITHELIAL CELLS

In contrast to the mesenchymal derivation of the framework in other lymphoid organs, the framework of the thymus is from the interaction between the ectoderm and endoderm. The exact contribution of each in the mature thymus is still a matter of discussion. Only the capsule, septa, trabeculae, and perivascular framework components in the thymus stroma are of mesenchymal origin. The major stromal cell component comprises reticular epithelial cells, which are characterized by the presence of tonofilaments and desmosomal connections. In the cortex the epithelial cells have a dendritic morphology, and extend with long slender dendritic cell processes in the parenchyma. In the fully developed thymus, the space in between these dendritic extensions is filled with lymphocytes. Even lymphocytes can be completely surrounded by the protrusions of a single epithelial cell, and exist in cytoplasmic vacuoles. These lympho-epithelial complexes, called thymic nurse cells, illustrate the close interactions between the epithelial microenvironment and develop-

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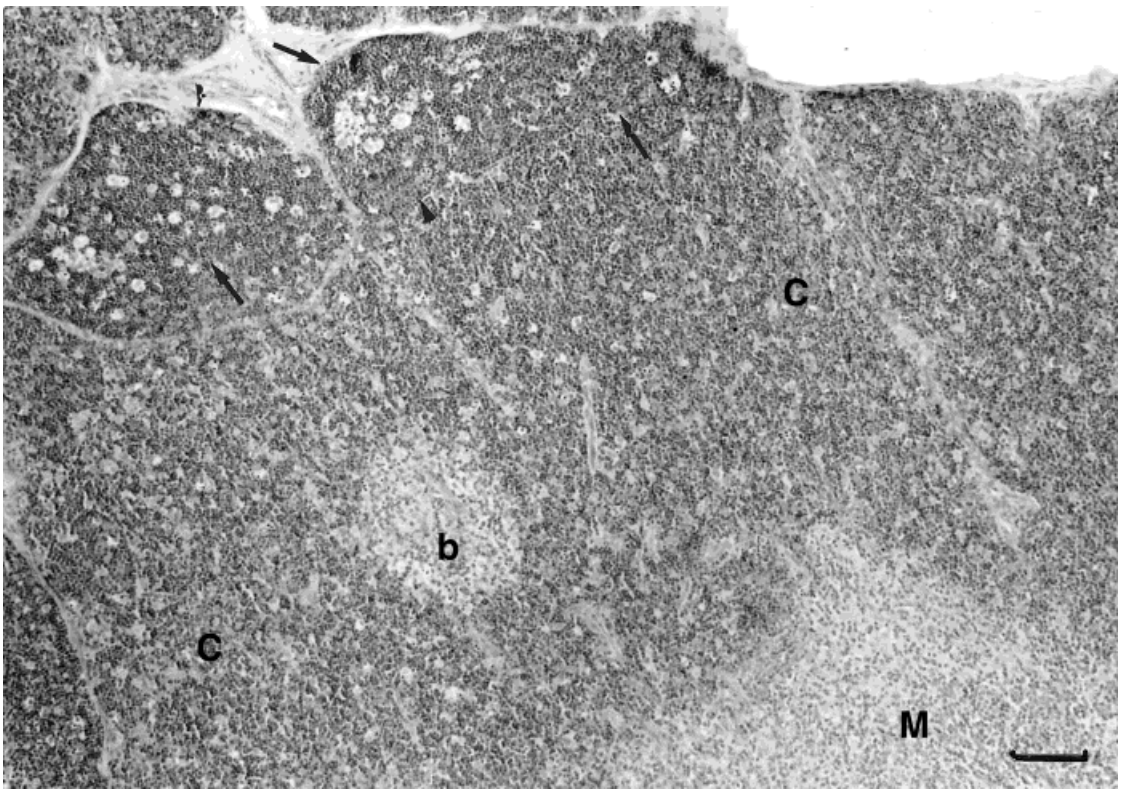

Fig. 1. Fully developed thymus in a young adult rat. Shown is a lobule, with cortex (C) and medulla (M). In the cortex there is a medullary bud (b). Note the presence of tingible body macrophages that give the cortex a starry-sky appearance; these tingible body macrophages are not immunolabeled by antibody ED2, and in part positive for antibody ED1 (shown in Fig. 2). Also shown are some epithelial-free areas (arrows). Formalin-fixed tissue, paraffin section stained with hematoxylin and eosin. Bar = 100 µm.

ing lymphocytes. They are not easily seen in conventional histology, but can be discerned in semithin (1 µM) sections and by immunohistology, e.g., for neuroendocrine markers such as oxytocin (Geenen et al., 1988; Wiemann and Ehret, 1993) and in the human thymus for Thy-1 (Ritter et al., 1981). It is still unknown whether these thymic nurse cells represent a special microenvironment in T-cell development in the cortex, or just represent the close contacts between cells at this location (Schuurman and Kater, 1985). In the medulla, the epithelial cells have a more rounded shape, with an excentric nucleus. In some species, a typical characteristic in the medulla is the presence of onion-like stromal structures, comprising large well-differentiated epithelial cells with centrally cellular debris, called Hassall's corpuscles (Kater, 1973). These are easily recognized in the thymus of, e.g., man and non-human primates, dog, pig, and guinea pig, but are less pronounced in the thymus of rats and most mouse strains. In these rodents, aggregates of epithelial stromal cells occur in the medulla, sometimes in a small circular orientation with some debris in the center. Hassall's corpuscles enclose cell debris and epithelial remnants, and are surrounded by large pale-staining epithelial cells.

Besides this cytologic heterogeneity in conventional histology, there is considerable heterogeneity within the epithelial compartment at the subcellular and phenotypic marker level. Six subtypes based on ultrastructural cytological features are distinguished in man (Van de Wijngaert et al., 1984), and this subtyping may be applicable in other species as well (De Waal et al., 1993; Kendall et al., 1988). Type 1 is the subcapsular/perivascular cell lining the capsule and vasculature with a basal lamina; dendritic-shaped cells in the cortex are differentiated into pale (type 2), intermediate (type 3), and dark (type 4) based on electron density of

Fig. 2. Some examples of immunohistochemistry to demonstrate various cell populations in a frozen thymus section of a young adult rat. In all cases the same area in the thymus is shown; cortex (C) and medulla (M) are indicated. **a,b:** Epithelial cells labeled by an antikeratin antibody recognizing all cytokeratin polypeptides (a) and antibody HIS-39 (b) (Kampinga et al., 1989), which reacts to subcapsular and medullary epithelial cells (CTES group II). Note the dendritic morphology of epithelial cells in the cortex, the epithelial-free area just underneath the subcapsular layer (arrow), and the more rounded shape of epithelial cells in the medulla. **c,d:** Macrophages labeled by antibodies ED1 (c) or ED2 (d) (Dijkstra et al., 1985). ED1 labels macrophages in cortex and medulla, and ED2 labels only a subset of macrophages in the cortex. Tingible body macrophages are not labeled by ED2. **e,f:** Lymphocytes labeled by antibody MRC-OX8 (CD8 cytotoxic-suppressor T-cell phenotype) (e), which labels all lymphocytes in the cortex and approximately one-third of lymphocytes in the medulla, and antibody R73 (f) reactive to a common epitope on the T-cell receptor, which labels thymocytes in the cortex in low intensity and lymphocytes in the medulla in high intensity. Note the presence of (more mature) scattered cells in the cortex that are labeled by this antibody in high intensity. Frozen tissue sections, indirect immunoperoxidase labeling (color development with 3,3-diaminobenzidine tetrahydrochloride and H_2O_2 and amplification with nickel ammoniumsulphate), with nuclear fast red counterstain. Bar = 100 µm. Unpublisged studies performed by R. Broekhuizen and H.-J. Schuurman, 1991, University Hospital, Utrecht, The Netherlands.

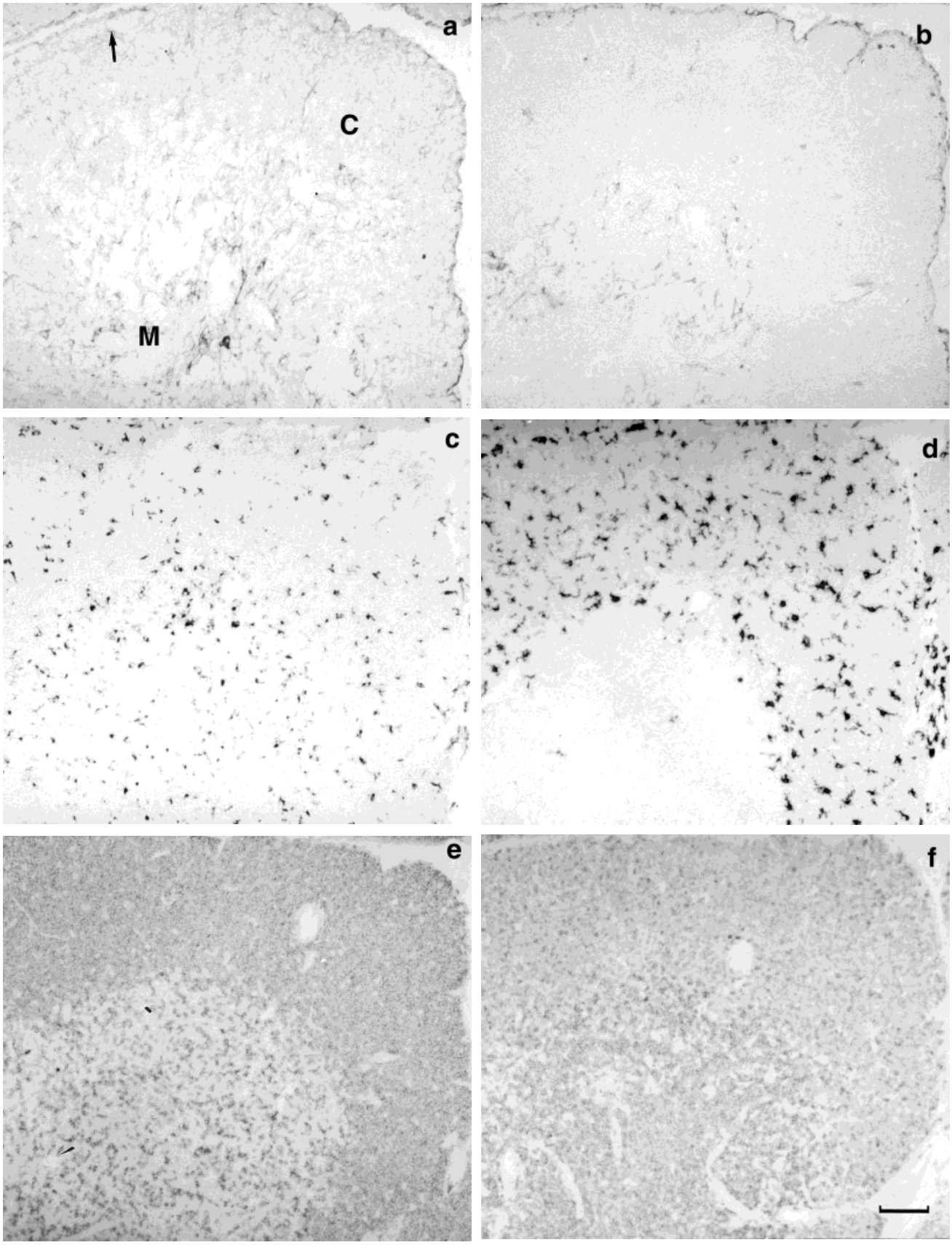

Fig. 2.

nucleus and cytoplasm; type 5 is a small undifferentiated cell that is present in low proportions in the medulla; and type 6 is a large secretory cell type in the medulla, amongst others surrounding Hassall's corpuscles. With some training, these cell types can be differentiated in conventional microscopy of semi-thin (1 μm) sections.

Also in phenotypic marker expression, heterogeneity is observed. A first differentiation can be made with anti-MHC class II antibodies, which in the human and rat thymus label the epithelial network in the cortex, but not or to a less extent epithelial cells in the subcapsular area and medulla (Schuurman et al., 1985; Von Gaudecker et al., this issue). With antibodies to cytokeratin polypeptides (Brekelmans and Van Ewijk, 1990; Colic et al., 1989; Farr and Braddy, 1989; Savino and Dardenne, 1988) a differential cytokeratin expression has been documented (Table 1). According to the cytokeratin expression pattern, the epithelium of the cortex appears as "simple" epithelium (expressing low molecular weight keratins), and that of the medulla as more "complex" (expressing high molecular weight keratins) (Brekelmans and Van Ewijk, 1990). Monoclonal antibodies have been generated to epithelial cell subpopulations in the human (Ritter and Haynes, 1987; Schuurman et al., 1987), mouse (Brekelmans and Van Ewijk, 1990), rat (Colic et al., 1988; Kampinga and Aspinall, 1990) and chicken (Boyd et al., 1992) thymus. These antibodies enabled various epithelial subtypes to be differentiated; an example is shown in Figure 2. In a multilaboratory approach, a grouping has been first made for antibodies to human epithelial cells (Ritter and Haynes, 1987) and subsequently extended to other species as well. This has resulted in the proposal of a "cluster of epithelial staining" (CTES) (Kampinga et al., 1989; Ladyman et al., 1991), that was recently revised by Boyd et al. (1993) (Table 2). A remarkable finding in these studies was the similar if not identical staining patterns by different antibodies to epithelium in different species (man, mouse, rat, chicken, axolotl); the CTES nomenclature holds in all species investigated. However, large variation existed between staining patterns by antibodies in an distinct CTES group when analysed on other lymphoid or non-lymphoid organs. This was documented in the evaluation of organs from the same species or from different species (unpublished data from the workshop).

Finally, a differentiation is evident based on the cytoplasmic expression of thymic hormones and neuroendocrine hormones. Epithelial cells in the subcapsular area and medulla are considered as hormone-synthesizing cells, and are easily stained with antibodies to, e.g., thymosin, thymulin, thymopoietin, and thymic humoral factor (Kendall and Stebbings, 1994; Von Gaudecker et al., this issue). But in the human and murine thymus, epithelial cells in the cortex can also be found positive for some hormones, with a heterogeneity in expression. This also applies to the expression of neural markers. Amongst others, this was demonstrated for the antibody A2B5, that recognises a complex ganglioside expressed by neurons, neural crest-derived and neuropeptide-secreting endocrine cells. In the thymus of man and rodents, A2B5-expressing cells are found in the subcapsular cortex, medulla, and around Hassall's corpuscles (Haynes et al., 1983). Other neuroendocrine hormones, like oxytocin, neurophysin, and vasopressin, have been demonstrated in similar staining patterns in the thymus (Geenen et al., 1988; Moll et al., 1988; Robert et al., 1992) (Fig. 3). Most neuropeptides are expressed by supcapsular/perivascular and medullary epithelium, although in the mouse thymus the cortex epithelium was also shown to be positive for oxytocin (Robert et al., 1992; Wiemann and Ehret, 1993). In the (outer) cortex microenvironment, thymic nurse epithelium apparently represents a distinct subset, as it shows expression of neuroendocrine markers (Geenen et al., 1988). Epithelial cells in the human thymus medulla have immunoreactivity for anterior pituitary hormones; and for some of these cortex epithelium also appears positive (Batanero et al., 1992). A final observation worth mentioning in this regard is the expression of epitopes of retroviral antigens by subtypes of thymic epithelial cells, mainly subcapsular/medullary epithelium (Parmentier et al., 1992; Schuurman et al., 1989).

TABLE 1. Cytokeratin markers on thymic epithelial cells[1]

Antibody	Cytokeratin recognised	Staining pattern
Human cytokeratins		
RCK 102	5, 8	Cortical epithelial cells and some medullary epithelial cells
RCK 103	5	Basal cells in several organs: in thymus to cortical epithelium and some medullary epithelial cells
RCK 105	7	Cortical epithelium and some medullary epithelial cells
RKSE 60	10	Hassall's corpuscles
Rat cytokeratins		
K 8.13	1, 5, 6, 7, 8, 10, 11, 18	Pan-epithelium
RPN 1162	7	Subcapsular/perivascular epithelium, part of epithelium in medulla
RPN 1166	8	Subcapsular/perivascular and cortex epithelium, part of epithelium in medulla
KL1	3, 10	Part of medullary epithelium, Hassall's corpuscles
K 8.12	13, 16	Subcapsular/perivascular epithelium, part of medullary epithelium
RPN 1160	18	Cortex epithelium, part of medullary epithelium
RPN 1165	19	Subcapsular/perivascular epithelium, part of medullary epithelium
Mouse cytokeratins		
34βE12	5, 6	Majority of medullary epithelium
35βH11	8	Subcapsular/perivascular, cortex, and medullary epithelium
RPN 1166	8	Cortex epithelium
RPN 1160	18	Cortex epithelium
KL1	3, 10	Subset of medullary epithelium
RPN 1165	19	Subset of medullary epithelium

[1]The numbering of cytokeratins is according to the classification of human cytokeratins (Moll et al., 1982). Antibodies are commercially available from Immunotech S.A. (Marseille, France), Organon Teknika-Cappel (Durham, NC), and Sigma Chemical Co. (St. Louis, MO).

TABLE 2. Epithelial subtypes defined by monoclonal antibodies: "cluster of epithelial staining" (CTES)

CTES	Specificity
I	Pan-epithelium
II	Supcapsular/perivascular and medullary epithelium
	Medullary network, Hassall's corpuscles
	Subcapsular/perivascular (II.A, thick; II.B, thin)
III	Cortex epithelium
	III.A, pan-cortex
	III.B, pan-cortex, infrequent subset medulla
	III.C, pan-cortex, subset leukocytes
	III.C1, macrophages
	III.C2, thymocytes
IV	Medullary epithelium, Hassall's corpuscles
V	Hassall's corpuscles
	V.A, Hassall's corpuscles
	V.B, Hassall's corpuscles, myeloid cells
	V.C, Hassall's corpuscles and associated medullary epithelium
	V.D, Hassall's corpuscles and associated medullary epithelium, subset of leukocytes
	V.E, Hassall's corpuscles and associated medullary epithelium, subcapsular epithelium
VI	Subcapsular (type-1 by electron microscopy) epithelium
XX	Miscellaneous
	XX.A, minority subcapsule, majority cortical and medullary epithelium
	XX.B, minority of cortical and medullary epithelium
	XX.C, minority medullary epithelium, cortical thymocytes

The phenotypic heterogeneity of the epithelium has been related to the developmental origin of the cells, which is not further discussed here. It might be related to the state of differentiation of the cells. This is illustrated by the differential expression of epithelial markers in the thymic anlage of mice with the severe combined immunodeficiency (*scid*) mutation. In this thymus anlage the (undifferentiated) epithelium only expresses markers of cortex epithelium; after reconstitution with lymphoid cells, the anlage develops into a fully "normal" thymus with cortex-medulla demarcation, including the differential expression of cortex or medullary phenotypes on the epithelial cells (Shores et al., 1991; Surh et al., 1992). A similar phenomenon has been observed for the thymic epithelial analage in mice that have been made lymphocyte-deficient by gene targeting (Boyd et al., 1993; Van Ewijk et al., 1994). The presence of Hassall's corpuscles surrounded by well-differentiated large epithelial cells is interpreted as reflecting functional activity of the thymus in this regard (Kater, 1973); in the inactive involuted thymus mainly hyalinised remnants of the corpuscles are observed (Huber et al., 1992; Schuurman et al., 1989). Also, the expression of neuroendocrine markers by thymic nurse cells in an otherwise negative cortex (mentioned above) points to such a differentiation process underlying phenotypic heterogeneity. Of importance here is that influences may come from mesenchymal "passenger" leukocytes that influence the organization of the thymic stationary microenvironment (Palmer et al., 1993). In vitro studies have shown that lymphocytes induce protein phosphorylation (Couture et al., 1992) and marker expression in cultured epithelial cells (Small et al., 1989). Interestingly, this epithelial differentiation can be induced not only by T cells bearing the αβ-T-cell receptor (representing the major population of lymphocytes in the normal thymus), but also by T cells bearing the γδ-T-cell receptor: in mice transgenic for a Vγ-T-cell receptor, Ferrick et al. (1990) even have observed a medullary hyperplasia. To describe these interactions between stationary and passenger cells in the thymus, Van Ewijk et al. (1994) have proposed the designation "crosstalk." Amongst others, cytokines (interleukins), pituitary hormones, and neuropeptides are involved in this crosstalk. These humoral factors, as well as receptors for these factors, are synthesized by thymocytes and thymic epithelial cells, and modulate both epithelial cell function and thymocyte differentiation (Dardenne and Savino, 1994; Ritter and Boyd, 1993). The existence of such a symbiosis between different cell populations was already known from studies on thymic involution, e.g., the "dedifferentiation" of epithelium and formation of epithelial rosettes in severely atrophic thymuses in patients with acquired immunodeficiency (Schuurman et al., 1989). Also histologic observations in thymomas, e.g., the existence of epithelium with medullary differentiation in thymocyte-rich "cortical" thymomas, is compatible with such "crosstalk" (Kirchner et al., 1989; Kuper and Beems, 1990).

In line with this "crosstalk," the heterogeneity of the epithelial stroma is generally ascribed to the function of the cells in various compartments of the thymus. Being a primary lymphoid organ, this function at first is to support and guide the differentiation of developing T (= thymus-dependent) cells. Knowledge of the molecular and cellular biologic events of T-cell processing inside the thymus has extensively increased during the last decade (Hedrick and Eidelman, 1993). Precursor T cells after arrival in the thymus first rearrange genes encoding T-cell receptor chains, and after expression of the T-cell receptor on the cell surface are selected in a positive and negative way before leaving the organ and contributing to the peripheral T-cell pool. Epithelial cells are considered as a crucial cell population directing this process. In the cortex, the cells express both class I and class II MHC antigens, and hence can promote positive selection of class I-restricted (CD8-phenotype, mainly precursors of cytotoxic T) cells and class II-restricted (CD4-phenotype, mainly precursors of helper T) cells. Also the cells express a number of autoantigens, and hence can effect the negative selection (deletion) of potentially autoreactive cells. Examples of such auto-antigens are retrovirus-encoded superantigens like M1s^a, and a similar role has been proposed for neuroendocrine hormones such as oxytocin and vasopressin (Robert et al., 1992). It is tempting to suggest that the expression of retrovirus-like antigens/epitopes also may be relevant in negative selection, but studies on this aspect have not been performed so far. Also, it is not known to what extent epithelial cells perform negative selection in vivo: interdigitating cells (describe below) might have a more important role in this respect.

Although this section deals with epithelial cells forming the supporting thymic stroma, areas within the thymus that lack such cells are also mentioned here. These so-called epithelial-free areas are easily identified in immunohistochemical staining, e.g., keratin (Fig. 2), and if aware of such areas, one can observe these too in conventional histologic sections (Fig. 1). So far, epithelial-free areas have been described in hu-

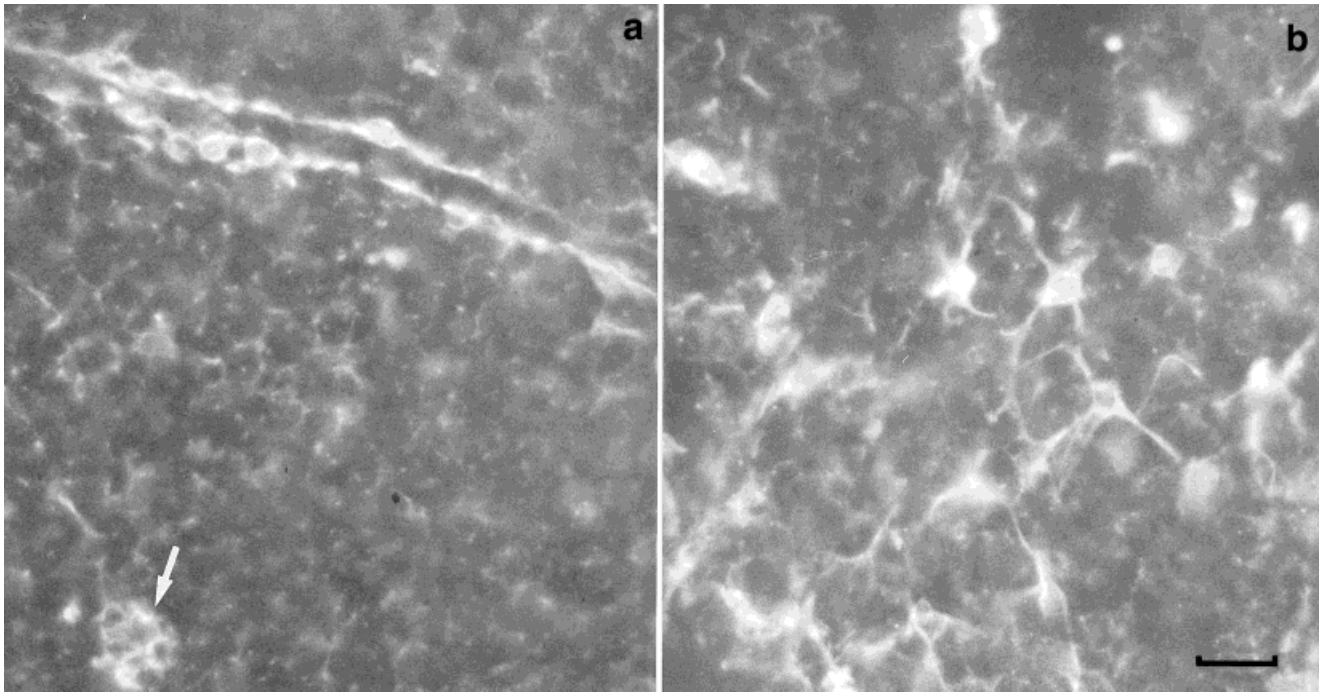

Fig. 3. Immunolabeling of frozen sections of a human pediatric thymus with antibody O33 to oxytocin (Robert et al., 1992). **A:** Cortex area of two adjacent lobules separated by a connective tissue septum, showing labeling of subcapsular epithelial cells (by electron microscopy defined as type 1 epithelial cells), and some cellular elements presumably a thymic nurse cell (arrow). **B:** Medulla area showing labeling of epithelial cells. Note the dendritic shape of the stellate epithelial cells. Indirect immunofluorescence, no counterstain. Bar = 40 μm. From unpublished studies performed by D.H.J. van Weering and M.D. Kendall, 1991, St. Thomas' Hospital, London, UK.

mans (Von Gaudecker, 1986), mice (Clarke et al., 1994; Godfrey et al., 1990), rats (Bruijntjes et al., 1993), and chickens (Boyd et al., 1992), and localized just beneath the covering subcapsular epithelial layer in the outer cortex. Kendall et al. (1990) have also observed these areas in the rat thymus, and at that time (mis)interpreted these as being perivascular areas. Epithelial-free areas can extend to deep in the cortex and reach the medulla. The areas lack the epithelial framework, but may contain some isolated reticular fibroblasts, and contain various macrophage subtypes that are strongly MHC class II positive. The areas are filled with small lymphocytes as in the thymus cortex, which can be in cycle as shown by in vivo bromodeoxyuridine labeling experiments (Bruijntjes et al., 1993). The significance of epithelial-free areas is not yet known. Apparently, they are not solely storage places for lymphocytes, as these cells can enter cell division at this location. Under some pathological conditions, like the pre-leukemic phase in the thymus of AKR mice, in the thymus of non-obese diabetic (NOD) mice, and the rat thymus after cyclosporine administration (Beijleveld et al., 1994; Bruijntjes et al., 1993), epithelial-free areas can be quite prominent. Epithelial-free areas are also seen in the medulla of the thymus, with extensions into the cortex environment (Clarke et al., 1994; Von Gaudecker, 1986). Such areas are particularly pronounced in the thymus of rats during recovery after cyclosporine treatment (Schuurman et al., 1990) and in the thymus of BB-rats (Rozing et al., 1989).

ACCESSORY CELLS OF BONE MARROW ORIGIN: MACROPHAGES AND INTERDIGITATING CELLS

Macrophages occur in the thymic cortex as well as in the medullary area. They can be distinguished in conventional histologic sections, although macrophages may resemble some cortical large lymphoblasts, and some epithelial cells can appear to have a similar morphology. In the cortex macrophages are easily distinguished by their large pale-stained cytoplasm filled with phagocytosed material, the tingible body macrophages (Fig. 1). In sections stained with hematoxylin and eosin, these macrophages form pale patches in the blue-coloured cortex, and therefore are also called starry-sky macrophages. The function of these macrophages is phagocytosis; they characteristically contain nuclear debris in the cytoplasm, presumably originating from lymphocytes after apoptosis (or programmed cell death) (Kendall, 1991). Increased numbers of tingible body macrophages, therefore, can indicate an enhanced apoptotic activity, for instance during acute corticosteroid-induced stress (Schuurman et al., 1992). Apart from a phagocytic function, macrophages may contribute to intrathymic precursor T-cell maturation. As with epithelium, close contacts with rosette formation can occur between macrophages and lymphocytes that show proliferation characteristics (Toussaint-Demylle et al., 1991). In this respect, the expression of MHC class II molecules can be related to antigen-presenting ability of the cells.

A classical method for a more detailed identification of macrophages is enzyme histochemistry, e.g., for acid α-naphthyl acetate esterase. This method is nowadays replaced by immunohistochemistry with specific anti-macrophage antibodies, especially since antibodies recognizing all macrophages have become available (Colic et al., 1990; Damoiseaux et al., 1989, Dijkstra et al., 1985) (Fig. 2).

Interdigitating cells, also called dendritic cells, belong to the monocyte/macrophage cell lineage, but follow a distinct differentiation pattern. In conventional thymus histology interdigitating cells are identified as large sized cells of irregular shape, with pale cytoplasm and an excentric nucleus. These cytologic features at the light microscopic level make it sometimes difficult to differentiate between large-sized solitary epithelial cells and interdigitating cells. Interdigitating cells are easily identified in tissue sections by immunohistochemistry. There are only a few antibodies that specifically recognize interdigitating cells, for instance in the rat the MRC-OX62 antibody presumably directed to a cell adhesion structure (Brenan and Puklavec, 1992), and in the mouse antibodies NLDC-8 and MIDC-8 (Breel et al., 1987). Antibodies to MHC class II antigen are often used in the identification of interdigitating cells, because the medullary interdigitating cells represent the main population that strongly expresses MHC class II at this location (Schuurman et al., 1985). Using MHC class II antibody to detect interdigitating cells, and anti-keratin antibody to detect epithelium, the thymus medulla normally shows aggregates of rounded epithelial cells, often in peripheral localizations near the corticomedullary zone as well as aggregates and Hassall's corpuscles scattered through the medulla, and large epithelial-free areas described above, which do not show a uniform expression of MHC class II antigen and presumably do therefore not contain interdigitating cells. The expression of MHC class II, as well as MHC class I antigen, has been directly linked to the function of the interdigitating cells, namely, negative selection of precursor T cells.

LYMPHOCYTES

Lymphocytes form the major passenger cell population of the thymus. In the sections stained with hematoxylin and eosin, these show varied morphologic features. Predominantly in the outer cortex but scattered throughout are large lymphoblastoid cells that morphologically are similar in size to subcapsular and outer cortical epithelial cells at this location. The cortex normally is densely packed with mainly small lymphocytes with scanty cytoplasm. Due to the dense lymphocytic population, neither cells of the stationary framework are easily discerned, nor are blood vessels. The exception is the population of tingible body macrophages mentioned above. In the medulla, medium-sized lymphocytes occur at a lower density, and here the stationary stroma, dendritic cells, and macrophages are more easily distinguished. The great majority of lymphocytes in the thymus are of the T (= thymus-dependent) lymphocyte lineage, but B cells and to a lesser extent plasma cells occur as well, especially in the medulla and perivascular spaces. Therefore, in contrast to the mainly primary lymphoid organ function of the cortex (that is, lymphocyte development irrespective of exogenous immune system stimulation), the medulla is considered as displaying both a primary and secondary immune organ function.

The cytology of lymphocytes is related to the immunologic phenotype of the cells (Fig. 2). Compared with other thymic cells, the phenotype and cytology of precursor T cells in the thymus have been closely associated with the function of the cells. Subcapsular lymphoblasts are most immature, small-sized cortex lymphocytes represent an intermediate maturation stage, and medullary lymphocytes the most mature stage in the thymus. In immunohistochemistry or flow cytometry using a wide spectrum of antibodies currently available, most of which have been grouped in clusters of differentiation (CD), a further subtyping becomes possible. The process that precursor T cells undergo during the intrathymic sojourn is nowadays described in molecular aspects of rearrangement of genes encoding the T-cell receptor, followed by positive and negative selection. These processes are reviewed in detail elsewhere (Hedrick and Eidelman, 1993) and have been related to the main stromal and accessory cell populations involved, e.g., epithelium in positive selection and interdigitating cells in negative selection. A scheme for the mouse thymocyte population is presented in Figure 4. This scheme includes the immunologic phenotype of different thymocyte subsets, the cytologic feature of the cells, their location in the thymus, and an indication of the main functional processes during intrathymic processing (receptor gene rearrangement, positive and negative selection). This scheme may apply for other species as well (Kampinga and Aspinall, 1990; Schuurman et al., 1993).

The scheme presented in Figure 4 essentially represents a crude simplification. By immunohistochemistry, cells with a mature phenotype not only occur in the medulla but are also scattered in the cortex (Fig. 2); cells with an immature "cortex" phenotype can easily be detected in the medulla. Considering positive and negative selection, there is now ample evidence that positive selection is not uniquely influenced by epithelial cells, and similarly negative selection is not a unique property of interdigitating cells (Elliott, 1993; Hugo et al., 1993; Martín-Fontecha et al., 1994; Robey and Fowlkes, 1994; Von Boehmer et al., 1993). The histologic location of the accessory cell involved, and its surface expression of relevant MHC molecules, with or without processed self-antigen, may be the first factors in mediating selection of thymocytes that are suceptible to processing. This phenomenon is a logical consequence of the hypotheses on "crosstalks" between developing T cells and stromal accessory cells described above.

Apart from epithelium and bone marrow-derived accessory cells, lymphocytes may interact with the extracellular matrix (Savino et al., 1993). This comprises a complex of multiple collagens, reticulin fibers, and glycosaminoglycans, which forms a fine network throughout the medulla and to a less extent in the cortex. In the thymic parenchyma, it is strongly associated with the vasculature. The subcapsular and perivascular epithelium is very close to the extracellular matrix, as it contributes to the stroma in trabecula and

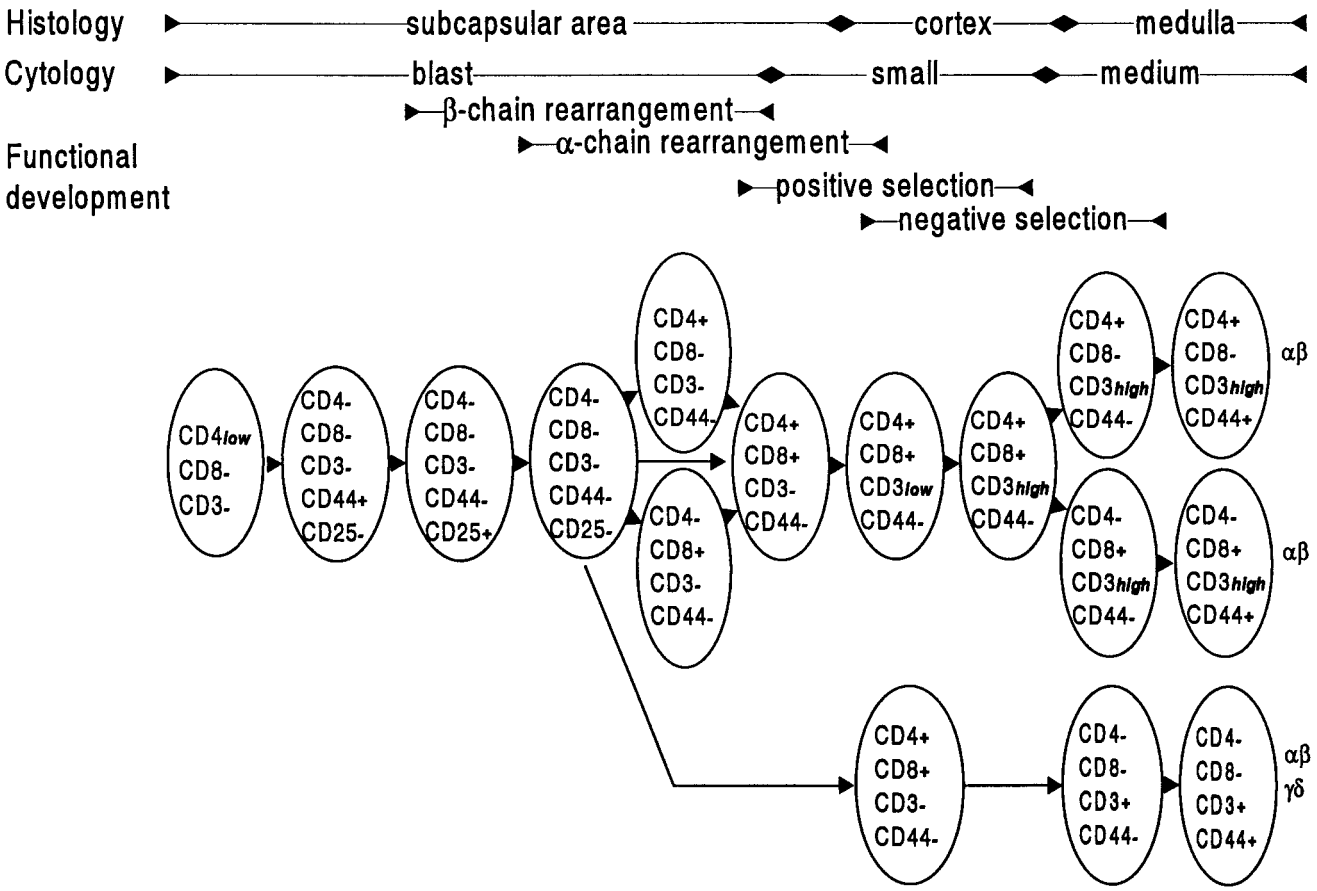

Fig. 4. Schematic representation of T-cell development in the mouse thymus. The developmental stages are indicated by the immunologic phenotype (CD4, helper-inducer phenotype; CD8, cytotoxic-suppressor phenotype; CD3, phenotype of cells expressing the T-cell receptor; CD25, cells expressing interleukin-2 receptors; CD44, cells expressing phagocytic glycoprotein-1, Pgp-1), in association with the tissue location, cytologic features, and stages in functional development (rearrangement of genes encoding the α or β chain of the T-cell receptor, positive and negative selection). The major lineage presented is the development of progenitor cells into mature $CD4^+8^-$ or $CD4^-8^+$ T cells with an αβ-T cell receptor. Also shown is the development into T cells bearing a γδ-T cell receptor, which is not further discussed in this review. Modified from P.J.A.M. Brekelmans, Ph.D., Thesis Erasmus University, Rotterdam, The Netherlands (1993).

perivascular spaces. Thymocytes in early developmental stages (e.g., the $CD4^-8^-$ or $CD4^-8^+3^-$ stage) express members of the CD29/49 β_1 integrin family, enabling the adherence to the extracellular matrix (e.g., fibronectin interacts with VLA-4, CD29/49d, and VLA-5, CD29/49e; laminin interacts with VLA-6, CD29/49f), and in addition to the epithelial stroma expressing the respective ligands (Cardarelli et al., 1988; Sawada et al., 1992; Utsumi et al., 1991; Wadsworth et al., 1993). Using such interactions, the extracellular matrix is thought to support migration of developing thymocytes; in addition a role in growth and development of thymus cell populations (lymphocytes and epithelial stroma) is ascribed to extracellular matrix proteins. This function may be related to the capacity to concentrate soluble cytokines to reach high local concentrations (Boyd et al., 1993).

This fluidity of intrathymic processes presents complications, when the histologic interpretation of thymus sections is aimed at a functional interpretation. This is even more complicated as not all cells in the section are in a distinct developmental transition stage; also the products of processes are not easily seen. Kinetic studies on the fully developed thymus in young mice have revealed that the evolution from immature prothymocytes till immunocompetent T cell takes about 3 weeks (Shortman, 1992). About 3% of the daily produced $CD4^+8^+$ cells develop into $CD4^+8^-$ or $CD4^-8^+$ cells and about 1% of the total thymocyte pool leaves the thymus each day in young animals (Shortman et al., 1990). Part of cellular activity can be observed in the conventional histologic section, such as lymphoblasts in the outer cortex, lymphocyte mitoses scattered through the cortex being most pronounced in the outer cortex and subcapsular area, apoptotic bodies dispersed through the cortex and medulla being concentrated in tingible body macrophages, and the presence of large well-differentiated epithelial cells and Hassall's corpucles in the medulla. The histologic picture does not appear to represent actual events, e.g., there is no massive cell death observed in histology, which may be a consequence of the fact that only a few thymocytes survive the selection process. One explanation for this apparent difference may be the speed of events including the removal of dead cells. Also, most of the lymphocytes observed in the histologic sections of the cortex may

actually represent sessile resting cells that are not participating in the maturation process. Concerning the medulla compartment, it is worth emphasizing that this area partly represents a secondary lymphoid organ, as it is accessible to exogenous antigens and lymphocytes, exemplified by the presence of B cells and plasma cells (Abou-Rabia and Kendall, 1994; Kendall et al., 1994). In the mouse, circulating T cells after activation can enter the thymus, and in the neonatal period this entry applies to resting T cells as well (Surh et al., 1993). Thus, the processes of a primary lymphoid organ, i.e., thymocyte differentiation not influenced by exogenous antigen stimulation, may occur at a lower level.

CONCLUDED REMARKS

The thymus is a dynamic organ, with a high degree of cell differentiation and proliferation. This not only is restricted to the lymphoid compartment. The sojourn of thymocytes, initially as prothymocytes, which results in mature T cells, is associated with complicated intracellular gene rearrangements and intercellular interactions during selection, and accompanied by cell proliferation, survival, and death. Accessory cells as macrophages and dendritic cells also show dynamic cell kinetics. For instance, the interdigitating cell population in the rat thymus is completely replaced within 3 weeks, which contrasts to the slow turnover of ED-2^+ cortical macrophages (Kampinga et al., 1990). Epithelial cells as well as interdigitating cells undergo mitosis (Van de Wijngaert et al., 1984) and differentiate into subtypes, which presumably correspond to differentiation stages from immature to fully mature or degenerating cells. The presence of well-differentiated epithelial cells in the medulla, as well as Hassall's corpuscles (Kater, 1973), has been associated with thymic activity.

The dynamic nature of the thymus can be illustrated by its fast response in terms of development and involution. In ontogeny, mice and rats manifest a fully developed thymus at day 18 of gestation, and in man this is the case at about 16 weeks of gestation (Von Gaudecker, 1986). This difference between species is related to the rather undeveloped immune system at birth of rodents, whereas that of humans is almost fully developed at birth. After birth, the human thymus shows a very fast weight increase, from about 15 g to 50 g within a few weeks. Throughout life, the thymus can show a rapid atrophy induced by exogenous stimuli, as well as age-related involution (Kuper et al., 1992; Steinmann, 1985). Seasonal changes in thymic histology in fishes, amphibia, birds, and some vertebrates have been reviewed by Kendall (1981). Physiological responses during pregnancy have been studied by Clarke and Kendall (1994) and Clarke et al. (1994).

The detailed observation and description of the various cellular components or their products, and the knowledge of the intricate interactions between different cell types, have been of value in the elucidation of the functional processes in the organ, although it should be emphasised that only a momentary picture of a dynamic process can be captured in sections. But with the interaction with modern biological tools and achievements in cell biology and molecular biology, the histology of the thymus is expected to continue to provide a crucial contribution to histophysiologic insights into the organ.

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