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Thymus and aging: morphological, radiological, and functional overview

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Abstract Aging is a continuous process that induces many alterations in the cytoarchitecture of different organs and systems both in humans and animals. Moreover, it is associated with increased susceptibility to infectious, autoimmune, and neoplastic processes. The thymus is a primary lymphoid organ responsible for the production of immunocompetent T cells and, with aging, it atrophies and declines in functions. Universality of thymic involution in all species possessing thymus, including human, indicates it as a long-standing evolutionary event. Although it is accepted that many factors contribute to age-associated thymic involution, little is known about the mechanisms involved in the process. The exact time point of the initiation is not well defined. To address the issue, we report the exact age of thymus throughout the review so that readers can have a nicely pictured synoptic view of the process. Focusing our attention on the different stages of the development of the thymus gland (natal, postnatal, adult, and old), we describe chronologically the morphological changes of the gland. We report that the thymic morphology and cell types are evolutionarily preserved in several vertebrate species. This finding is important in understanding the similar problems caused by senescence and other diseases. Another point that we considered very important is to indicate the assessment of the thymus through radiological images to highlight its variability in shape, size, and anatomical conformation.

Keywords Aging · Human · Rodent · Thymus

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Introduction

Aging is a continuous and slow process compromising the morphofunctional characteristics of different organs and systems both in humans and in animals (Rose et al. 2012). Moreover, it is associated with a decline in the normal functioning of the immune system that is described by the canopy term "immunosenescence" (Dixit 2012). The latter claim is gathering interest in the scientific and healthcare communities alike since the increasing aged population poses new challenges to healthcare systems.

Immunosenescence is generally and popularly related with weaker immune responses which produce a progressive deterioration in the ability to respond to new stimulants (Bauer et al. 2009; Yan and Wei 2011). The updated view is a remodeling of several immunological components inducing reorganization and changes in many organs. This remodeling has been observed both in adaptive immunity and in innate

immune functions during aging and mainly observed in centenarians. In fact, it shows intact adaptive or innate immune functions and is a good example for successful aging (Franceschi and Bonafè 2003).

The immunosenescence is responsible for increased susceptibility to infectious diseases, neoplasia, and autoimmune diseases (Castle 2000). The exact mechanisms involved in immunosenescence are not fully understood, but one of the important causes is the regression or involution of the thymus.

The thymus is a primary lymphoid organ responsible for the production of a diverse repertoire of immunocompetent T cells. The first demonstration of its crucial role in establishing the development of a normal immune system was provided in 1961 when it was shown that mice thymectomized immediately after birth had poorly developed lymphoid tissues, impaired immune responses, and inordinate susceptibility to intercurrent infections (Miller 2002). This important finding has been obtained by Miller during his Ph.D. degree in a shack at a place called Pollards Wood in Chalfont St. Giles, about 1 h by train from Greater London (Fig. 1). However, with age there is a well-defined and accepted decline in the thymus size and function (Fig. 2) (Gui et al. 2012). Regression of the thymus leads to a decline in naïve T cells output modifying the composition of peripheral T cells pool and altering T cells phenotype and function (Aw and Palmer 2012). These changes are believed to significantly contribute towards the clinical features of immunosenescence (Aw et al. 2007). It is important to remember that age-associated thymic involution occurs in humans as well as in many other species that possess a thymus, indicating this as an evolutionary ancient and conserved event (Torroba and Zapata 2003).

Fig. 1 The shack at Pollards Wood in Chalfont St. Giles in which Miller obtained his important finding about thymus and its crucial role in establishing the development of immune system working on neonatally thymectomized mice (Miller 2002)

Although it is accepted that intrinsic and extrinsic factors may contribute to age-associated thymic involution, little is known about the mechanisms concerning thymic involution. There are many unanswered questions as to what initiates this process and when exactly it begins. Thus, increasingly our understanding of the potential mechanisms believed responsible for thymic involution and optimizing interventional strategies for restoring thymic structure and function may help to maintain the correct immune system in aging. At this aim, this review focuses on our current understanding of thymic structure in young and elderly underlying: (1) the architectural changes and (2) the possible alterations of cellular markers and their archetypal staining pattern. Moreover, we considered the thymic morphology in several diseases that are not only linked to aging, such as obesity and diabetes. This is to better understand the anatomical changes linked or due to these diseases. It is known that obesity, diabetes, and cardiovascular diseases are the primary risk factors for health care.

In the literature, thymic involution is scarcely and inadequately studied. We sought to better clarify the thymic question by reporting the timesheet of the thymus to have the same start point. By measuring the natal, postnatal, adult, old, and very old in this manner, we have a clearer understanding of morphological changes in the thymus. We considered it noteworthy to report, in one paragraph, that the thymic morphology and cell types are evolutionarily preserved in several vertebrate species. This finding is important in understanding the similar problems caused by senescence and other diseases. Another point that we considered very important has been to report the evaluation of and radiological images for the thymus to highlight its variability in shape, size, and anatomical conformation.

Difference between human and rodent thymus

The basic structure and cellular types are evolutionary preserved in different vertebrate species that show the same organization of lymphocyte and non-lymphocyte populations in cortex and medulla. This finding occurs despite the fact that human connective tissue septa are more abundant and open

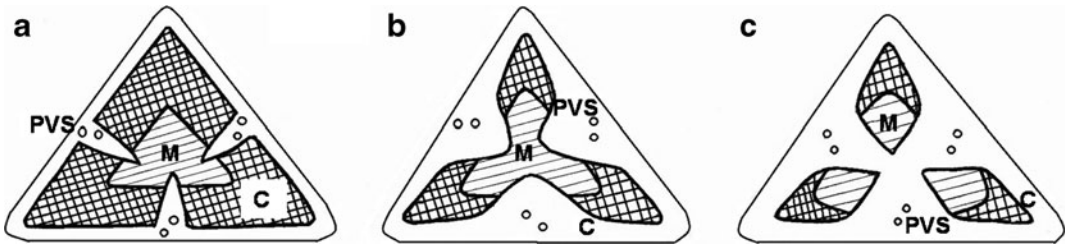

Fig. 2 A schematic representation of parenchymal thymus changes in years. Different representation of medulla (*M*), cortex (*C*), and perivascular space (*PVS*) in 1- (**a**), 25- (**b**), and 75-year-old (**c**) subjects

at the ends. These open-end septa branch the perivascular spaces (PVSs) in the corticomedullary bundle. The PVSs in the corticomedullary junction (CMJ) are intermingled with the epithelial compartment. However, they are separated from it by a layer of thymic epithelial cells (TECs) called cortical epithelial cells (cTECs) (von Gaudecker 1997; von Gaudecker et al. 1997) which are normally present in the cortex. Furthermore, a population of metallophillic macrophages is typically found in mice but not in humankind (Milićević et al. 1983; Milićević and Milićević 1984). Hassall's bodies (HBs), structure found in the medulla of the thymus, are large and composed of many TECs in humans; in mice however, they are small and made up of only one or few cells.

Definition of natal, postnatal, adult, old and "very old" thymus in humans and rodents

First of all, it is important to underline that the human thymus develops during fetal life, reaches its maximal output during early postnatal life, and declines in size and output during young adulthood and throughout adult life through the process of age-related involution (Chinn et al. 2012).

Parallels between T cells kinetics in mice and men have reinforced the idea that a young mouse is a good model system for a young human, an old mouse, for an elderly human, and very old mice for old human (den Braber et al. 2012; Stämpfli et al. 2010). For mice, we define the young "postnatal mouse" period as the time from birth to 6–8 weeks of life. We define a "baby" mouse as 1–2 weeks old, an "old" mouse as starting from 12 to 14 months, and the intercurrent stage corresponding to adulthood between 6 and 8 weeks to 12 months (Koo et al. 1982; Miller et al. 2002). A 76 weeks old aged mice corresponds approximately to a 60- to 65-year-old human. A "very old" mouse that is older than 104 weeks corresponds roughly to a 70- or 80-year-old human (Bulckaen et al. 2008; Stämpfli et al. 2010).

At our knowledge, there are many studies carried out in old rodents but few data or no findings are found in "very old" animals. Thus, we reported separated results for postnatal, adult, old and "very old" thymuses, when they were available.

Thymic anatomy and its cytoarchitecture

The embryological origin of the thymus, the number of thymus organs per animal and the anatomical positions of lobes are different in all species. For example, chickens have seven, sharks five, and amphibians three thymus, while mammals, humans, and mice included, have only one thymus composed of two bilateral lobes (Boehm and Bleul 2007). The new three-dimensional techniques, introduced by Irla et al. (2013), have allowed to describe in mouse model a large central medulla connected or surrounded by smaller islets of medulla tissue, but for humans there is a more detailed description of thymus in literature. In other words Irla et al. establish the existence of an intricate central compartment that is either subtly connected to hundred small areas of medulla. With three-dimensional reconstruction, it is possible to calculate the mean volume of medullary areas about 1.6×10^{-4} mm^3. Another important evaluation is the distance between cortical area and CMJ that is about 114±42 μm.

It is becoming increasingly clear that the thymus and its specialized microenvironments play a key role in the multiple developmental processes leading to the generation of functionally mature T cells (Anderson et al. 2000).

The thymus consists of two pyramidal lobes, connected by areolar tissue, and enclosed in a fibrous capsulae dividing them into lobules. It is located in the mediastinum behind the sternum and in front of the

pericardium and great vessels of the heart. The primordial of thymus develops in the region of the superior neck in the early fetal life and reaches its final destination in the mediastinum by progressive descent (Krishna and Subhadra 2012). During the descent, the thymus can be implanted along the cervical pathway and appears afterwards as an ectopic thymus.

The literature reports several anatomical variations regarding number of lobes, presence of ectopic tissue, accessory lobes and extension of thymus. Krishna and Subhadra (2012) carried out a study on a sample (n=53) of human thymus glands collected from pre- and post-natal cadavers of different groups. The thymus glands were recorded on a wide range starting in prenatal period to 65 years of age. The authors found that all prenatal and two of the postnatal thymus glands were pyramidal in shape. The pyramidal thymus glands were observed in less than 16 years age group. The other studied thymus glands were flat; the colors of the thymuses were pinkish grey to brown in prenatal period and whitish grey to yellow in postnatal period. Moreover, the Authors demonstrated that: a high number of thymic samples had two lobes and a low number had three or four lobes and that there are respectively eight and eleven different types of gross anatomical variations (Table 1) and cervical extension (Table 2).

The individual lobules, as well as the thymic lobes, are variable in size, shape, and orientation. The histological elements of the thymus are quite similar in all vertebrates.

The thymic microenvironment consists in a network of different cells known as the thymic *stroma* (Taub and Longo 2005) and is composed of two regions defined as *cortex* and *medulla* (Fig. 3). Thymic stroma can be viewed as all nonhemopoietic components of the thymus that are functionally defined as those elements. Regardless of their origin and lineage, these components constitute the thymic structure and improve a matrix on which thymocytes develop (Fig. 4). A classification of stroma is based on keratin expression in that keratin$^+$ cells, and keratin$^-$ cells are a mixture of mesenchimal cells. Keratin$^+$ cells are composed of two major subsets, referred to as cTECs and medullary TEC (mTECs). Keratin$^-$ cells are considered mesenchimal cells and include fibroblasts, non-fibroblastic mesenchimal cells, capsule- and septae-forming connective tissue cells, and endothelial cells forming the typical thymus vasculature (Anderson et al. 2000; Gray et al. 2007; Rodewald 2008). Finally, HBs, dendritic cells (DCs) and macrophages, and CD45$^+$ hemopoietic cells are also important elements of thymus stroma.

Table 1 Distribution of thymus anatomical variations (modified from Krishna and Subhadra 2012)

	Anatomical featues	Cases reported
1	Cervical extensions of thymus	40
2	Accessory lobes	14
3	Fibrous band connection to thyroid	10
4	Accessory thymus	7
5	Thymus extension up to diaphragm	5
6	Thymus above thyroid	3
7	Feathery margins	1
7	Thymus behind innominate vein	1

Table 2 Type of thymus cervical extensions (modified from Krishna and Subhadra 2012)

	Different kind of cervical extension	Cases reported
1	Up to right lobe of thyroid gland	9
2	Up to suprasternal notch	8
3	The lower pole of thyroid gland	7
4	Isthmus of thyroid gland	5
5	Above suprasternal notch	4
6	Up to middle of the neck	2
7	Left lobe of thymus above thyroid	1
7	Right lobe of thymus up to thyroid and left lobe above thyroid gland	1
7	Thymus extending above left thyroid cartilage	1
7	Thymus up to the isthmus of thyroid in midline and left lobe up to left thyroid cartilagine	1
7	Up to left lobe of thyroid gland	1

The cellular heterogeneity of thymic structure and that of many organs makes it more difficult to study the development or function of cell types in the physiologic context and their transient or permanent contribution to the thymus structure. We believe that this review reporting the changes and markers of thymus cellular heterogeneity, from a morphofunctional point of view, can help in the understanding of this enigmatic organ.

Ectopic thymus

Although thymic tissue normally has a mediastinal location, some studies report a casuistry of cervical

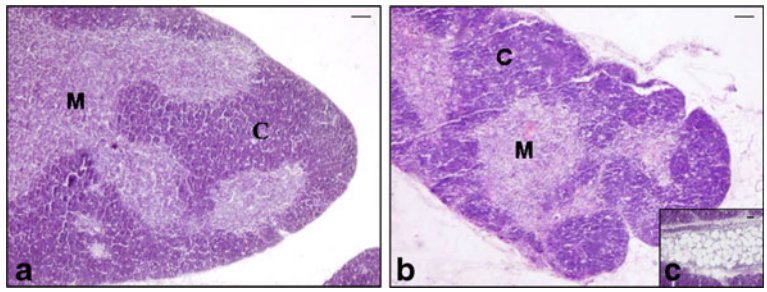

Fig. 3 Photomicrographs of young (**a**) and old (**b**) mice thymus showing a change in cortical/medulla ratio. It is important to note that the septa in old mice thymus are fulfilled by adipocytes (**c**), demonstrating the thymic degeneration with age. *M* medulla, *C* cortex. Hematoxylin–eosin. *Bar*, 20 μm

thymic tissue that can be considered rare (Büyükyavuz et al. 2002), despite Zieliński et al. (2004) have demonstrated an incidence of cervical thymic tissue in humans around 50 %. The ectopic thymus, in both humans and mice, reflects a failed migration of thymic tissue from third pharyngeal pouch endoderm during the organogenesis. It may be found at any level of the pathway of normal thymic descent, from the angle of the mandible to the superior mediastinum. It can be considered as a sequestration of thymic tissue during descent or a failure of involution. Ectopic thymus is usually located anteriorly and deep to the middle third of the sternocleidomastoid muscle, adhere posteriorly to the carotid sheath and often extend into the retropharyngeal space. Approximately 50 % of all cervical thymic masses. may be continuous with the mediastinal thymus by direct extension or by connection to a vestigial remnant or a solid cord (Saggese et al. 2002).

In mice, cervical thymic tissue is rarely bilateral, situated in the left or right position with a cephalic–caudal orientation. It is located in a range from immediately above the sternum to association with the thyroid and parathyroid glands. The ectopic tissue functionally resembles the thoracic thymus in terms of stromal cell composition and organization. Moreover, the structure preserve the abilities to support thymocyte development and to contribute to central tolerance. Histological demonstration of what appeared to be cervical thymic tissue in mice was suggested to explain the failure of some strains of mice to display reductions in peripheral lymphocyte cellularity following neonatal thymectomy (Law et al. 1964; Miller et al. 1962).

There are many papers showing case reports the ectopic thymus in humans. Mostly of that cervical position of the organ can make a compression on the closest structure of the neck. In other cases, the ectopic tissue is misdiagnosed as lymphadenopathy or neoplasia and only a biopsy and radiology confirm the connection to the thymus, although the diagnosis is usually in the postoperatory (Ahsan et al. 2010). Ahsan et al. underline that ectopic cervical thymus is a rare anomaly and 50 % of cases occur in children. In a study conduct on 12 child patients, the ectopies are unilateral

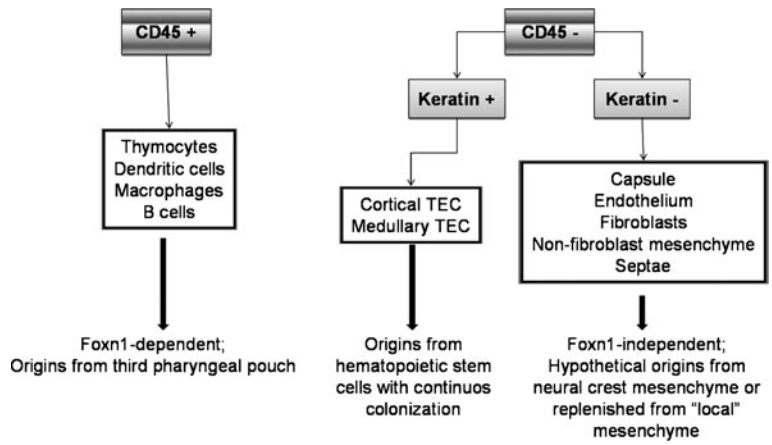

Fig. 4 Thymus major cell types and their developmental origin (modified from Rodewald 2008)

in nine and bilateral in three patients. All the cervical thymus are located at the mid- to lower portion of the thyroid and the long-axis diameter of the ectopy has a mean of 1.5 cm. All ectopic thymus have a fusiform shape with well-defined margins (Kim et al. 2012).

Cell thymic overview in physiological conditions

In the following paragraphs, we describe the different types of cells and their cellular interactions underlying the morphological and functional differences in human, rodent of natal, postnatal, adult, and old thymus.

Origin and development of thymic epithelia cells

The thymus originates from endodermal cells of the ventral third pharyngeal pouches during early gestation. In the last period of gestation, there is differentiation of mTECs and cTECs. After birth, the replication of the all types of thymic cells continues until reaching a "steady state," in which the TEC population does not have any expansion or loss. This occurs despite the fact that thymic epithelium shows morphological changes and reduction starting from the 6th month in mouse models and reaching the maximum alteration after the 24th month.

The classification of mTECs and cTECs is important because they are morphologically and functionally different. mTECs are indispensable in the establishing central tolerance with the expression of autoimmune regulator gene and in the promoting the development of $CD4^{+}CD25^{+}Foxp^{3+}$ T regulatory and natural killer (NK) T cells (Manley et al. 2011), while cTECs are involved in positive and negative selection (Goldman et al. 2005).

TECs are known to be a dynamic population with rapid turnover (Gray et al. 2006). It is necessary to have a pool of stem cells that regenerate lost cells to maintain this framework. Thymic epithelial stem (TESC) or progenitor cells (TEPC) in the fetal thymus fulfill these important roles by building up the thymic epithelial lineage starting from fetal period. The presence of these cells is demonstrated in an experiment in which forkhead-box transcription factor (Foxn1) is blocked. Consequently, TEC lineage cells undergo maturational arrest and persist as progenitors (positive for two antibodies MTS20 and MTS24) (Blackburn et al. 1996). TECs are phenotipically homogeneous until embryonic day (ED) 11.5 mice, at which point Foxn1 starts its expression (Gill et al. 2002; Bennett et al. 2002). Dealing with the development of the medulla, the participation of two precursor cells of distinct phenotypic traits cannot be excluded (Naquet et al. 1999; Rodewald et al. 2001; Brelińska et al. 2002a). We summarized the markers in Table 3.

TECs in postnatal thymus

TESC/TEPC lineage persists also during the postnatal thymus. It was provided by an analysis of a subset of human epithelial tumors with subpopulations of cortical and medulla suggesting a common origin from epithelial stem cell (Schluep et al. 1988).

For the following description, it is important to note that the postnatal thymus is populated mostly by TECs with the double expression of cytokeratins 5 and 8. This population gives rise to a differentiation in cTEC $K5^{-}K8^{+}$ (Klug et al. 1998). Moreover, the possibility to regenerate TECs persist into adulthood. In fact, it has been demonstrated that exogenous stimulation with keratinocyte growth factor stimulates TECs proliferation, restoring the normal capacity of thymopoiesis and thymic architecture (Min et al. 2007).

During the first week of postnatal life, increased proliferation is associated with a predominance of cTECs in contrast to the mTECs predominance of the adult thymus. The quantification of cytokeratins K5 (expressed by mTECs) and K8 (expressed by cTECs) expression allowed the analysis of the ratio cTECs/mTECs, which is 1.78 in the neonatal thymus and 0.56 in adults (Gray et al. 2005); this underlies the large number of cTECs in relation to mTECs population in postnatal mice. This situation is reversed in the

Table 3 List of markers that vary during age in mice, summarizing the data of the authors cited in the paragraph "TECs in old thymus"

	Development	Adult mice	Old mice
Aire	++	+	+/–
Foxn1	++	+	+/–
MTS20	++	+	+/–
MTS24	++	+	+/–
Cytokeratin 5	++	+	+/–
Cytokeratin 8	+++	++	+
Cytokeratin 10	+	+	+/–

old thymus in which the number of mTECs was increased. This difference is seen in both humans and rats (Brelińska et al. 2002b).

It is known that the interaction between T cells, TECs and many receptors is important to establish the final thymic layout, starting from the fetal period. During embryogenesis, T cell progenitors accumulate at the outer mesenchymal layer and, after the EC 11, CCR7, CCR9 and CXCR4 play an unique role in the migration inside the parenchyma of T cells (Gray et al. 2005).

TECs in adult thymus

Considering medulla and cortex separately, it is possible to identify different subpopulations of TECs during steady state: types I, II, III, and IV in cortex and I, II, III, IV, V, VI, and VII in medulla (Milićević and Milićević 2004; Rezzani et al. 2008) (Fig. 5).

Type I TECs "Subcapsular" TEC that are located in the connective tissue and always have a basal lamina. They have an irregular shape and their prolongations have desmosomes to interconnect cells. The nucleus is euchromatic and the cytoplasm has an active framework of keratin tonofilaments and vacuoles scattered throughout the entire cytoplasm. Golgi complexes and endoplasmic reticulum are well developed. They usually showed around them a variable numbers of thymocytes ranging from one to less than 100. In particular, in the medulla the position of type I TECs is located around capillaries.

Type II TECs "Pale" TEC that are present in outermost areas of thymus. They have the shape of stars with short prolongations. The nucleus is widely large, euchromatic with nucleoli and shows low electron density. The cytoplasm has a high cellular activity with low electron density. It is filled with a lot of vacuoles resembling a foam like appearance and it a contains high number of ribosomes. Tonofilaments are mostly in the perinuclear cytoplasm and more represented in cytoplasmic prolongations. They are considered "nurse cells" because they meet and follow thymocytes during their development inside the thymic parenchyma. This type of cell shares a number of features with type I.

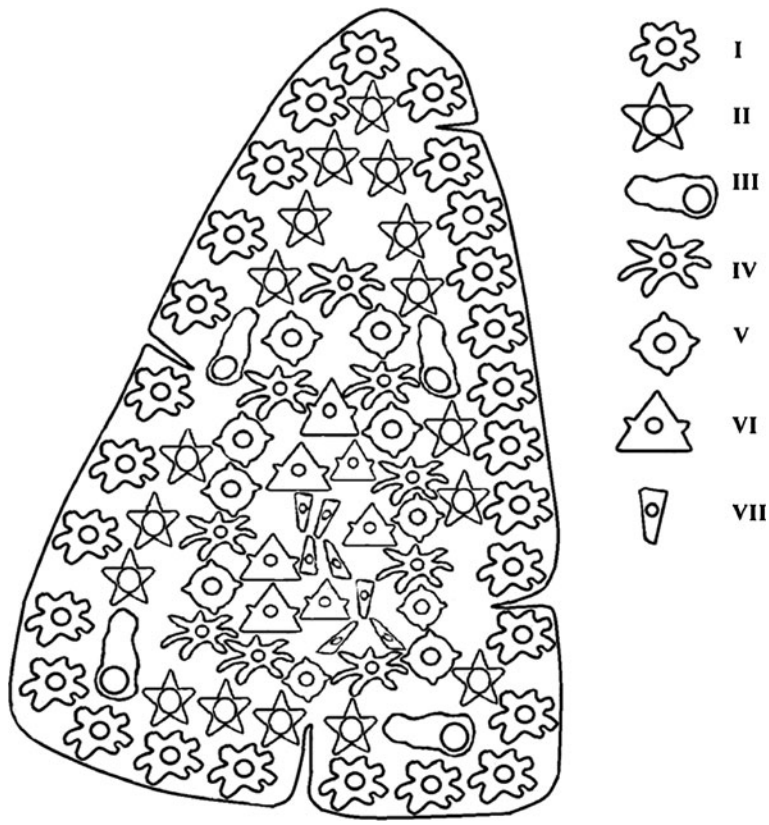

Fig. 5 A schematic representation of the different subpopulation of TECs and their most commom position inside thymus parenchyma

Type III TECs "Intermediate" TEC that are located in deeper cortex. They are infrequent and formed by single TECs. The cells are polarized in respect to the positions of organelles. The nucleus is smaller, heterochromatin is dispersed and its position is in one of cytoplasm poles. The cytoplasm is abundant with sheet-like extensiona and fulfilled with organelles like vacuoles. Tonofilaments are mostly present in cytoplasmic prolongations. The cytoplasm also contains mitochondria, electron dense granules, electron lucid vesicles of variable size and vacuoles with smooth walls or walls covered with individual microvilli. This type of cell has a higher electron density cytoplasm and nucleus in comparison with type II cell.

Type IV TECs "Dark" TECs that are positioned in the deep cortex and at the CMJ where they can penetrate the medulla. Heterochromatin is spread in the nucleus and nucleoli are really prominent. Cytoplasm extensions are very long and contain many organelles like the cell body. There are secretory vacuoles and lipid droplets. Keratin bundles are present in the whole cytoplasm. The whole cell shows a high electron density.

Type V TECs "Undifferentiated" TECs that are most located in the CMJ. They have round shape and short prolongations with a blastoid appearance. The electron density is low and cytoplasm shows few tonofilaments and polyribosomes.

Type VI TECs "Large medullary" TECs that have abundant cytoplasm and, few cytoplasmic extensions. There is an abundance of transport vesicles, dilated profiles of endoplasmic reticulum, large Golgi and large vacuoles underlining the high metabolic activity of these cells.

Type VII TECs "Spindle-shaped" TECs that are small and arranged in groups or connected with each other by large desmosomes. The cytoplasm is fulfilled of cytokeratin with scanty organelles (Milićević and Milićević 2004; Rezzani et al. 2008).

In humans, it is described only TECs are polyhedral or starshaped, with many cytoplasm extension, creating an extracellular network for the development of T cells (Cavallotti et al. 2008).

TECs in old thymus

Cavallotti et al. (2008) showed that in human thymus extracted from young and elderly patients, a deep change in the framework of the organ. Structurally, aged thymuses displayed a large mass of adipose tissue containing scattered thymic islands composed of TEC, lymphocytes, and reticular connective tissue (Cavallotti et al. 2008¸ Hannestad et al. 1997). In particular, Cavallotti et al. (2008) estimate that TECs are 50.5 conventional units (CU) in young humans and 41.6 CU in old thymus. As the ageing progresses, epithelial cells decrease in number and show cystic changes and reduced intracellular granules (Mocchegiani et al. 2006; Fabris et al. 1997).

Hirokawa et al. (1983) observed the activity of 5′-nucleotidase in the aged thymus at the level of TECs covering folliculoid mass of thymocytes, showing quite similar pattern to that of the cortex of the young thymus, and occasionally in the aggregated mass of the TECs. Although, adenosin triphosphatase (ATPase) was occasionally demonstrated in the agregated mass of TECs of human aging thymuses and macrophages, Hassall's corpuscles and cortical thymocytes were negative for this enzyme. Moreover, Hirokawa et al. (1983) observed, during age-related disintegration of the thymic architecture, weak activity of the enzyme acid phosphatase in the TECs arranged in aggregated mass of various size and in those lining inner surface of the cyst probably transformed from Hassall's corpuscles. These findings suggest that an aging, involuting thymus is still playing a role in the maintenance of the immune system by producing either T cells or thymic factor of small amount.

Hirokawa et al. (1983) observed also an age-associated decrease of absolute number of thymosin α_1-positive cells, potent inducer of helper T cells, in consistent with reports that serum level of thymosin and thymosin-like factors decrease with age and the thymic activity to promote T cell differentiation declined with age-related thymic involution.

Nerve growth factor (NGF), the best-characterized member of a family of neurotrophic factors collectively known as neurotrophins, is a molecule with a wide spectrum of biological functions, including regulation of the immune system (Levi-Montalcini et al., 1996). Marinova et al. (2003) observed NGF- and NGF receptor-immunopositive TEC in senile human thymus and supported the hypothesis that NGF and/or NGF

receptors might be implicated in thymus functions mediated by TECs. Moreover, Hannestad et al. (1997) observed, in both rat and human thymus, that TrkAIR, the essential constituent of the NGF high-affinity signal transducing receptor that may also serve as a receptor for other neurotrophins, mainly neurotrophin-3 (NT-3), and might be indicative NGF or NT-3 activity, was mainly localized in the mTEC subtype and only in some cases cTECs and so the authors concluded that TrkA ligands NGF and NT-3, presumably acting in an autocrine or paracrine manner, may be involved in the control of TECs. This could be of potential importance because of the role of TECs in providing an appropriate microenvironment for maturation and selection of thymocytes.

More details in literature are reported about the morphological changes that are different between cortex and medulla in mice and we considered them in the following paragraphs.

Cortex

The first stage of alterations due to involution starts between the 12th and 15th month of mice life (when they are considered adults prone to age) and is characterized by an extensive cytoplasm vacuolisation, dilatation of endoplasmic reticulum and infiltration of lypofuscin material. In a second stage starting from the 15th month of mice life (when they are considered old). mTECs and cTECs lose their intra-cytoplasmic differentiation, losing organelles and vacuoles. It is possible to find cytoplasmic syncytium containing lipofuscin material, lipid inclusion, thymocytes, and few organelles (Nabarra and Andrianarison 1996). Buckland et al. (2000) and Ortman et al. (2002) showed a reduction of biological activity of cTEC upon thymocytes.

Medulla

In old mice, a great variety of hormonal factors acts on mTEC activity with an up- or downregulation of target cells. An example of down-regulation is present on mTEC with cytokeratin 10; the slow disappearance with age of cytokeratin 10 in mTECs corresponds to an up-proliferation of subcapsular TECs. This produces the disorganization of thymic cytoarchitecture with the formation of cystic cavities filled with PAS-positive material (Takeoka et al. 1996; Brelińska and Warchol 1997). The consequence of this proliferation determines a thymic morphology showing a pseudo-follicle type with a basement membrane present only on the surface of cells in contact with connective tissue. In addition, the most common markers become gradually attenuated or disappeared.

In all populations of TECs, there is a modification of the autophagic activity, a physiological process of self-degradation of cellular components. In the thymus, this aspect plays a role in the presentation of self-antigens, built up to form cellular peptides. After the 24th month this function is greatly decreased and immunofluorescence can detect autophagosome with LC3 expression; 50 % is the reduction in 12-month-old mice and another 50 % of decrease in 24-month-old mice (Uddin et al. 2012).

Cell interaction

There is a strong dialogue between thymocytes and TECs. In fact thymocytes stimulate the production of interleukin-6 (IL-6), an autocrine growth factor in culture, by TECs. This was evidenced by the act that a combination of anti-IL-6 and anti-IL-6 receptor antibodies caused 70 % inhibition of TEC proliferation. At the same time transforming growth factor-β (TGF-β) inhibits the TEC growth in culture (Meilin et al. 1995).

Another important interaction is the presence of tight junctions (TJ) in the mTEC network that allows the interaction between DCs and thymocytes. The main function of this interaction is the transferring of small molecules. Their expression is under the control of p53 family members (Ichimiya and Kojima 2006). The lack of p53 conducts to an histological abnormalities of thymus, due to the deep link to TJ (Celli et al. 1999). Furthermore, p53 is connected to the regulation of the expression of Notch receptors that are indispensable to induce T cell precursor to differentiate into mature T cell in mouse models (Laws and Osborne 2004).

Dealing with the early constitution of thymic blood vessels and, the production of vascular endothelial growth factor (VEGF), which are fundamental for this process, is demonstrated in TECs and thymocytes, especially during the postnatal period in mice. This is connected with the vascular architecture formation of neonatal thymus. The level of VEGF slowly decreases in the adulthood, reaching approximately zero in old

specimens (Cuddihy et al. 2009). While in humans, Fedorova et al. (2009) observed a minimum quantity of VEGF in thymus of advanced age.

Anderson et al. (2000) demonstrated the presence of a local growth factors (fibroblast growth factor-7 (Fgf-7) and fibroblast growth factor-10 (Fgf-10)) produced by vascular elements and embryonic mesenchymal cell, which are required to shape the adult thymic layout. Fgf-7 and Fgf-10 are expressed in the mesenchyme surrounding the thymic epithelial primordium and Fgf-10-deficient mice also exhibit impaired thymic growth. Fgf signaling is essential for thymic epithelial proliferation. This has a repercussion on the generation of thymocytes and their very low numbers, although the maturation of T cells continues from the CD4/CD8-double-positive to CD4/CD8-single-positive stages (Revest et al. 2001). Moreover, in association with the development of thymic vascular tree, some mTECs grow together; their proliferation leads to the formation of islets of medullary precursors in the early state of constitution (Anderson et al. 2000).

Thymic DCs

TDCs play a central role in shaping and maturating T cells by deleting self-reactive thymocytes to establish central tolerance and by starting the development of regulatory T cells. They have a common multipotent precursor which has also the opportunity to differentiate in T cells, B cells, and NK cells (Varas et al. 2003).

Thymic DC origin

TDCs are members of bone-marrow-derived DC family and develop inside the thymus (Savchenko et al. 2006). They have a dendritic shape and are found mainly at the thymic CMJ and in the medullary region (Lafontaine et al. 1997). The function of TDC is generating central T cell tolerance through the deletion or negative selection of autoreactive thymocytes (Ardavín et al. 2001). There are two subpopulations of DCs, lymphoid and myeloid, that are possible precursors of TDCs (Shortman and Caux 1997). A model integrating the myeloid and lymphoid origins of DC is reported in Fig. 6.

Lymphoid-related DC

The evidence that TDCs have lymphoid origin comes from studies carried out on mouse thymic DCs and on a subgroup of DCs present in spleen and in lymph nodes (Vremec et al. 1992; Wu et al. 1995). In particular, the original evidence for lymphoid-related DC came from cell transfer studies using purified T cell precursors. Other approaches have reinforced this concept. It is important to remember that, although the CD4 T-precursor population is unable to produce macrophages in cell transfer studies, it is still an efficient precursor of DC, likely to lymphocytes (Ardavin et al. 1993). Moreover, these studies showed that the cells reported above express several markers normally associated with lymphoid cells, such as CD28, CD2, and CD45.

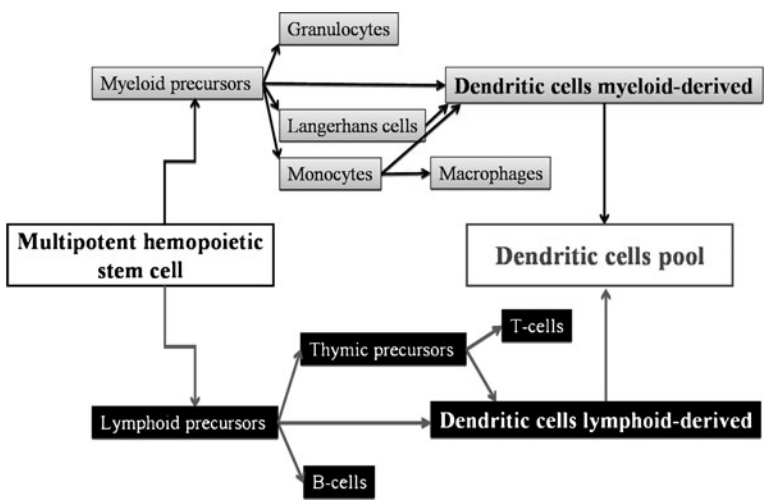

Fig. 6 Different possible pathways for dentritic cell formation (modified from Shortman and Caux 1997)

Myeloid-derived DC

The concept that DCs are of myeloid origin and that they are closely related to macrophages received strong evidence when they were generated in culture from peritoneal cells (Rezzani et al. 1999). In this study, our group demonstrated that cells obtained from mouse peritoneal cavity lavage can be induced to differentiate in vitro along the dendritic lineage by the addition of optimal concentrations of murine recombinant granulocyte macrophage colony stimulating factor (GM-CSF). Moreover, Rezzani et al. (1999) demonstrated that the peritoneal cells were induced to differentiate into macrophages by treating in vivo the animals with thioglycollate before peritoneal harvesting. These cells completely lost the ability to acquire in vitro the dendritic phenotype in response to GM-CSF, either used alone or in combination with tumor necrosis factor-α (TNF-α). These data suggest that resting peritoneal cells in the mouse represent an immature population, capable of further differentiation along either the dendritic or the macrophagic pathway, depending on the type of stimuli they receive.

Thymic DC in natal thymus

TDCs start to appear in the thymic mouse tissue at the 14th ED. The total numbers of TDCs increase with age thereafter until 5th week of age approximately. The increase of total amount of DC has paralleled the increase in the number of total thymocytes (Dakic et al. 2004). The analysis of thymic DC for surface expression of CD8α, CD205, and MHC class II molecules demonstrate that TDC from 17th ED and 1-day-old mice are mostly CD8α^{-}. CD8α^{+} TDC appears in the thymus of 1-week-old mice (approximately 30 % DC) and becomes the majority of thymic DC (70–80 %) by 2 week of age, at which time the phenotype resembled that of TDC in adult mice. At all stages, all TDCs are CD205^{+} and CD4^{-} (Vremec et al. 2000).

At the 4th month of human fetus, the thymic cytoarchitecture is not well distinguished. Instead a specific marker of TDCs (S 100) is shown at the CMJ and in the medulla (Savchenko et al. 2006). These two areas represent the most common area where TDCs take place. Starting from the 5th month of gestation cortex, medulla and HBs become apparent and Langerin (CD207)-positive cells can be observed around HBs, close to TECs. The positivity to the Langerin marker is typical of Langerhans cell-like present in thymus. Initially Langerin-positive cells are located only nearby HBs and afterwards they are found in CMJ and medulla. Inside these cells there are high density granules widespread in cytoplasm, which have the name of Birbeck granules (BGs). In the 8th month of gestation, Langerin-positive cells colonize the lumen of lymphatic vessels.

Electron microscopic detection of BGs in the cytoplasm of TDC and immunohistochemical detection of Lag positivity in TDC clearly indicate that TDCs express also a Langherans-cell-like phenotype during human ontogeny. Other studies carried out by Kissenpfennig et al. (2005) have shown that Langerin is also a potent inducer of BGs. BGs arise from the cell membrane by an endocytic pathway that results in the capture and subsequent intracellular routing of material into BGs (Valladeau et al. 2000).

Thymic cortical dendritic macrophages in postnatal thymus

A particular type of macrophage called thymic cortical dendritic macrophage (TDCM) was found in humans (Wakimoto et al. 2008). TCDMs usually contain varying numbers of apoptotic thymocytes, although lacking in lysozyme, CD68 and major histocompatibility complex class II (MHC-II) molecules. Moreover, TCDM express some DC-associated molecules including fascin, an actin-binding protein specific for DCs. TDCMs are strictly closed to the capillaries and they cover with their protrusions the small vessels inside the parenchyma. They have remarkable electron-lucent and abundant cytoplasm with many tubulovescicular structures and secondary lysosomes. In contrast to the TCDM, ordinary macrophages (OM) do not contain condensed nuclei of apoptotic thymocytes at all. These findings strongly suggest that TCDM, but not OM, plays a central role in the clearance of apoptotic thymocytes in the human thymus. Moreover, it has been demonstrated that OM are frequently found to contact with TCDM, which result suggests that OM cooperate with TCDM in the digestion of phagocytized nuclei of apoptotic thymocytes. Interestingly, such cooperation between phagocytic macrophages and other thymic stromal cells has been reported (Ezaki and Uehara 1997; Samms et al. 2001). When the TCDM fixed macrophages and these latter are unable to migrate, they may have to seek, capture, and phagocytize

neighboring apoptotic thymocytes through their dendrites (Fig. 7).

TDCMs have dendritic or plump features. Plump TDCMs usually contains a lot of degraded nuclei, while dendritic TDCMs contains only one. In particular, Wakimoto et al. (2008) studied the ultrastructure of dendritic TCDMs from normal human thymuses of patients with heart diseases at the time of heart surgery. The ages of the patients are 6-months and 6 years (Fig. 8). They showed that TCDMs from patients 6 months are easily identified as large cells with abundant, electron-lucent cytoplasm, membrane invaginations and cytoplasmic projections around thymocytes (Fig. 8a). They have a slightly euchromatin-rich nucleus with several nucleoli and tubulovescicular structrures, several lysosomes and occasional apoptotic thymocytes. They were frequently around the vessels and their projections showed apoptotic thymocytes (Fig. 8b,c). Moreover, the Authors showed that in the patients 6 years, plump TDCMs are identical to dendritic TDCMs from the other studied patients; they are large cells with electron-lucent cytoplasm containing many nuclei of apoptotic thymocytes and lysosomes (Fig. 8d). These cells are positive to fascin and ultrastructural analysis showed that they have electron-lucent cytoplasm with apoptotic thymocytes and, as above reported, a number of lysosomes (Fig. 8e). Thus, the ultrastructural features of fascin-positive cortical cells are similar to those cells identified by electron microscopy. An immune electron microscopic study for lysozyme, which is detected in lysosomes, showed that OMs are smaller than TCDM and have small lysosomes but not apoptotic thymocytes (Fig. 8f).

On the basis of these data, it is important to note that dendritic TCDMs resembles myeloid dendritic cell (mDC), showing electron-lucent cytoplasm, numerous tubule-vescicular structures, and membrane invaginations. Since mDCs express self-antigens on their surface and are involved in negative selection of thymocytes and TCDM phagocytize many thymocytes, these

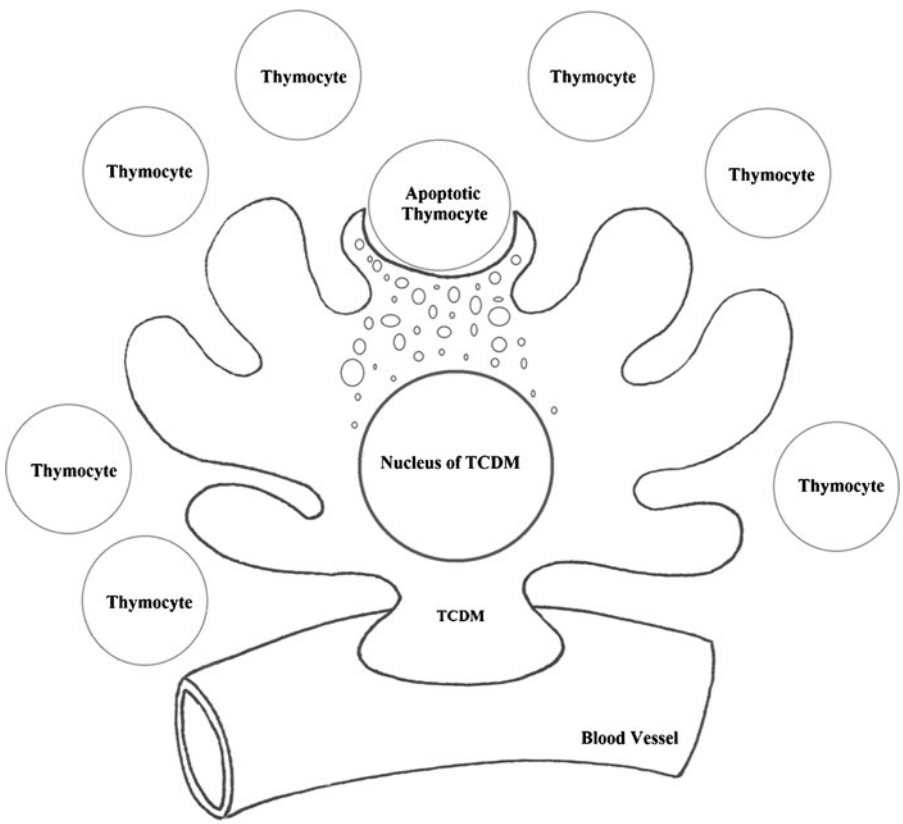

Fig. 7 A schematic representation of a thymic cortical dendritic macrophage that phagocytes an apoptotic thymocyte

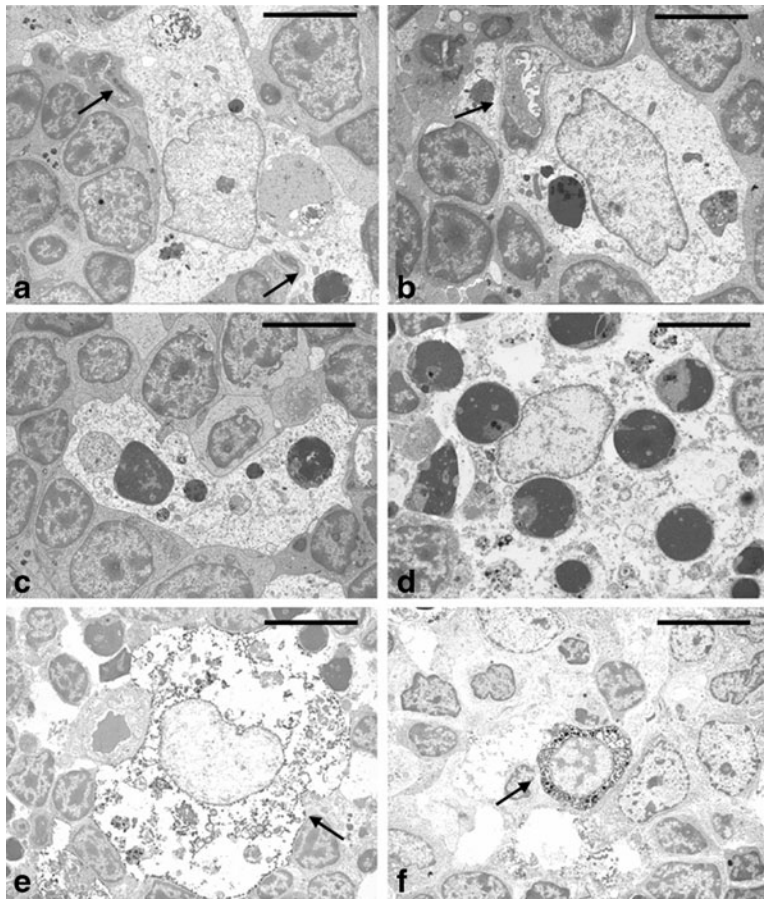

Fig. 8 Electron microscopy photomicrographs of thymic cortex from a patient 6-months old (**a**–**c**) and a patient 6 years old (**d**). *Arrows* indicate small capillaries. Immune electron microscopy photomicrographs for fascin of the thymic cortex from a patient 6 years old (**e**; *arrow* indicates fascin) and for lacking lysozome of the thymic cortex from a patient 6 years old (**f**; *arrow* indicates lysozome). Original magnification, ×2,500 (**a**, **c**), ×3,000 (**b**), and ×2,000 (**d**). (modified from Wakimoto et al. 2008)

Authors propose that these cells could be rich in self-antigens involved in tolerance induction.

Thymic DCs in adult thymus

In mice, the $CD11c^{+}CD45RA^{-}$ TDC represent 0.2–0.5 % of the total thymic cells, when some thymocytes has gone through the selection processes (Dakic et al. 2004). Although the proportion of DC in the thymus overall is only around 0.5 %, lower than in other lymphoid organs, within the medulla itself the incidence of DC would be comparable to that in the spleen or lymphnodes (Wu and Shortman 2005). After the differentiation, TDCs have an interdigitated shape with abundant and electron lucent cytoplasm. Many agglomerated lysosomes are located in the perinuclear cytoplasm and BGs. The cytoplasmic protrusions have a close relationship with the membrane of thymocytes. The nucleus has both an atypical and characteristic shape (van Haelst 1969; Ardavín 1997).

Thymic DCs in old thymus

Although it is demonstrated that there is an aged-related reduction of DC population, the proportion of human thymic DCs remains constant between postnatal, adult and old humans as in mice (Varas et al. 2003). Their cytoplasm appears clarified, reinflated and empty as if having lost most of the cellular components. There may be a correlation between that involution and the loss of efficiency in the T cells selection.

Thymic plasmacytoid DCs

About 35 % of the DC lineage cells in the thymus are the plasmacytoid DCs (pDC) which closely resemble the pDC found in peripheral lymphoid organs. These pDC can be differentiated from a DC-enriched preparation as $CD11c^{medium}$ MHC II^{low}, $CD45RA^{high}$, and $CD45R^{high}$ cells. Thymic pDC have a plasmacytoid rather than dendritic morphology but transform to dendritic morphology on activation in culture (Wu

and Shortman 2005). In a new-born mouse, pDC represent 0.04 % of the total thymic cells and grow up in parallel with TDC. At the 6th week of age, the total amount of pDC in thymus is less than TDCs pool (Dakic et al. 2004).

Macrophages

The origin of macrophages has not been well established yet. However, two hypotheses were formulated about their origins. In particular, one hypothesis shows that the monocytes enter into thymus through the PVS and afterwards differentiates in macrophages (Sminia et al. 1986). A more recent hypothesis proposed the presence of a common cell progenitors in the thymus that can also give rise to T cells, DCs, B cells, and NK cells (Lee et al. 2001). Macrophages are type of thymic stromal cell that are involved in phagocytosis, antigen presentation and producing cytokines, which influence T cells proliferation and maturation (Milićević et al. 1987). There are not many differences across natal, postnatal, and adult thymus and very few information is known about old thymus. At our knowledge, in literature are available many papers about thymic macrophages in mice models, but no about humans. The most represented population of mouse thymic macrophages has shape of DC with well-developed processes, spreading in the adjacent areas surrounding T cells. These types of macrophages are placed in the all thymus regions and are positive for two antibodies: anti-F4/80 and anti-Mac-2 (Liu et al. 2013). A brief discussion about the difference between cortical and CMJ macrophages, we reported only the modifications in old thymus.

Cortical macrophages

They have flat shape with scanty cytoplasm that are uniformly widespread in mouse thymic cortex, especially in subcapsular region. Cortical macrophages are stained by anti-Mac-2, but they are not stained by anti-F4/80 antibodies (Liu et al. 2013). With the help of electron microscopy, it is possible to see dead lymphocytes at several stages of degradation (Milićević et al. 1987).

Corticomedullary macrophages

They are small oval cells that lacked cell processes, making a garland between cortex and medulla, although they can be found in the middle of medullary region. Anti-Mac-2 antibody stains strongly that subpopulation of mice macrophages, while anti-F4/80 antibody stains weakly these cells (Liu et al. 2013). They contain cytoplasmic inclusion filled with oxidized lipids and cholesterol (Milićević and Milićević 1984). The nucleus is euchromatic with prominent nucleolus and many Golgi complexes. The presence of phagocytized lymphocytes is not so common. Finally, in the context of mice corticomedullary region a small numbers of irregular shaped macrophages is detected by Liu et al. (2013), and they are positive for anti-F4/80 antibody.

In Table 4, we summarize the mice expression of F4/80 and Mac-2 in the different macrophage subpopulations.

Aging and macrophages

With time the macrophage population undergoes a decrease with a reduction of precursors and their capacity to proliferate (Zeira and Gallily 1990).

Conflicting results have been reported on the age-dependent changes of monocyte/macrophage functional capacities. In detail, while the features of adherence and chemotaxis are increased in old thymus (Ortega et al. 2000), there are several results showing a different phagocytic capacity in old macrophages. Some authors, in fact, showed that this capacity is diminished or unchanged in age subjects, while others have found an increased phagocytic activity of these cells during old age (Ortega et al. 2000). In addition, the capacity of antigen-presenting seems to be reduced or unchanged. At this purpose, decreased levels of MHC II molecules have been described in activated macrophages from aged mice (Xaus et al. 2000) and in monocytes from elderly human subjects (Varas et al. 2003).

Table 4 Schematic representation of F4/80 and Mac-2 in the different macrophage subpopulations

	Cortex	Corticomedullary region	Medulla
F4/80	−	+	−/+
Mac-2	+	−	++

T lymphocytes

Nowadays, it is clear that the immune system decline leads to a progressive deficiency in infection response. This aspect is due to a conspicuous reduction of naïve $CD8^+$ T cells and at the same time an increase of effector $CD8^+$ T cells in old humans. The amount of memory T cell is constant in one's lifespan as is the production of naïve $CD4^+$ T cells and the quantity of effector $CD4^+$ T cells in young and aged people (Ferrando-Martínez et al. 2011). $CD8^+$ T cell pool is more affected by age-related changes than $CD4^+$ T cell pool. This reduction was studied investigating CD57 expression levels and shortened telomeres that are two markers of replicative senescence. This loss is connected with two phenomena: the first reason is the reduced capacity of bone marrow to generate lymphoid progenitors due to deficiencies of DNA damage repair and the shortening of telomerase. The second reason is the atrophy of thymus, which leads to a drop in development of T cells. Cavallotti et al. (2008) estimate thet the total T cell population in young human is about 36.1 CU and it decrease in old age reaching 17.6 CU.

T-lymphoid precursor cells colonize the thymus on ED 12–14 in a mouse-model (Douagi et al. 2000) under the effect of unknown chemokines. This migration from the periphery is shown also in the adulthood but the mechanism remains to be shown. In mice there are two waves of colonization peacking, one between the 10th and 13th ED and the second one between the 18th and 21th ED. T cells enter via the post-capillary venules (PCVs) at the CMJ. Despite the lack of knowledge about the colonization the most of signals (CCL19 and CCL21) and receptors (CCR9, CCR7, and CXCR4) concerning with the migration inside thymus are known. The maturation process lasts between 3 and 14 days and at the end single positive (SP) thymocytes emigrate from thymus to the periphery. T lymphocytes exit from the thymic parenchyma as recent thymic emigrants (RTEs).

Thymus and the cells inside it are important for the development of thymocytes starting from the double negative (DN) to SP T cells. This mechanism makes a positive and negative selection to create an active T lymphocyte that respects the tolerance of self-antigens. At first, T cells are DN, in the second stage they are double positive (DP) and finally SP in the third stage.

An ultrastructural analysis of mouse thymocyte subpopulations showed five different kinds of T cells inside the organ (Abe and Ito 1970; Yagi et al. 1997; Rezzani et al. 2008).

Double-negative thymocytes ($CD4^-8^-$) are the most immature T cells in thymus of mice. They show as large size and irregular shape with abundant cytoplasm and widespread organelles. The nucleus is irregular and eccentric with well spread euchromatin. This subpopulation represents 5 % of total thymocyte population and

Table 5 Ultrastructural differences of thymocyte subpopulation (modified from Yagi et al. 1997)

Small $CD4^+8^+$	$CD4^+8^-$	$CD4^-8^-$	$CD4^-8^+$ type I	$CD4^-8^+$ type II	Large $CD4^+8^+$ type I	Large $CD4^+8^+$ type II
Small dimension	Medium dimension	Large dimension	Medium dimension	Large dimension	Large dimension	Large dimension
Round-shaped nucleus	Round-shaped nucleus	Irregular-shaped nucleus with eccentric position	Round-shaped nucleus	Irregular-shaped nucleus with eccentric position	Round-shaped nucleus	Irregular-shaped nucleus
Heterochromatic chromatin	Heterochromatic chromatin	Euchromatic chromatin	Heterochromatic chromatin	Euchromatic chromatin	Heterochromatic chromatin	Euchromatic chromatin
Round cellular shape	Round cellular shape	Irregular cellular shape	Round cellular shape	Irregular cellular shape	Round cellular shape	Round cellular shape
Smooth plasma membrane	Uneven plasma membrane	Rough plasma membrane	Uneven plasma membrane	Rough plasma membrane	Smooth plasma membrane	Rough plasma membrane
Scanty cytoplasm		Abundant cytoplasm		Abundant cytoplasm	Scanty cytoplasm	Abundant cytoplasm
Poor cell organelles	Cell organelles	Widespread cell organelles	Cell organelles	Widespread cell organelles	Poor cell organelles	Widespread cell organelles

it is localized inside the subcapsular region, within the microenviroment of subcapsular TECs.

Small double positive thymocytes ($CD4^+8^+$) are the predominant subpopulation reaching the level of 80 % of thymocytes. They are small cells with round shapes, scanty cytoplasm, a not well-developed Golgi apparatus and few mitochondria. The nucleus is round with chromatin organized in clumps. Small DP and large DP thymocytes occupy the mid- and deep-cortical regions with a triple interaction with dark, intermediate, pale TECs.

Large double positive thymocytes ($CD4^+8^+$) are divided into two types by the contrast of chromatin in nucleus. Type I has scanty cytoplasm and rare organelles with a round heterochromatic nucleus. Type II has abundant cytoplasm, many organelles and an irregular euchromatic nucleus. Both types have a round shape. These cells represent 10–15 % of thymocyte population.

CD4 SP thymocytes ($CD4^+8^-$) have intermediate and spherical sizes with round heterochromatic nuclei. The Golgi apparatus is moderately developed and mitochondria are widespread in cytoplasm. They make up 10 % of all thymocytes.

CD8 single-positive thymocytes ($CD4^-8^+$) have two morphological patterns. Type I has an intermediate size and round shape with a moderately developed Golgi apparatus and mitochondria. In this case, the nucleus is round and appears heterochromatic. Type II has a large size with an irregular shape, abundant cytoplasm and well-developed organelles. This subpopulation represents approximately 5 % of thymocytes.

Gui et al. (2007) showed that the thymus has a normal recruitment of lymphohematopoietic progenitor cells (LPCs). The recruitment inside the organ has the same intensity in young and old mice due to the similar level expression of P-selectin and CCL25 by thymic cells. These molecules are supposed to be linked to attract LPCs. Although the number of LPCs recruited is more or less the same, the total amount of T cell precursor in aged thymus is considerably reduced. There is a correlation between the architectural and phenotypical modification inside the thymic stroma and the reduction of T cell development. The medullary region presents improvement in apoptosis of thymocytes, compared with new born mice.

In analyzing the output wave of RTEs, it is possible to observe that RTEs are phenotypically and functionally distinct in neonatal and adult mice (Opiela et al. 2009). In addition, the total amount of these cells is more copious in young than adult mice. RTEs during neonatal period are able to produce higher levels of Th effector cytokines in comparison with adult mouse (Table 5).

Hassall's bodies

Although it is known that HBs are involved in both maturation of developing thymocytes and removal of apoptotic cells, their exact action remains an enigma. They are considered as the unique components of the thymus, which provide developing thymocytes with paracrine and juxtacrine signals to help their functional maturation during the intrathymic lymphopoiesis (Marinova et al. 2009).

HBs in natal, postnatal, and adult thymus

Regarding the appearance of HBs, there are many controversies. In the following paragraphs, we reported data and observation from human studies. At our knpwledge there are few studies in animals, in particular, Ebbesen and Christensen (1972) analyzed the HBs in rodents. These Authors, studing the thymus of guinea pig, observed that HBs of oestrogen-treated animals appeared larger than those of untreated animals. Furthermore, the HBs contained a few Foa–Kurloff cells (FK), considered as lymphocytes and as macrophages (Kittas et al. 1979). The main organs of production of FK are considered thymus or the spleen; however, it has been shown that the rate of formation of FK cells is not influenced by thymectomy (Ranlov et al. 1970; Kittas et al. 1979). Moreover, the study of Kittas et al. pointed to FK as a possible source of a thymus-humoral factor released from the HBs.

Berthelot et al. (2010) showed that HBs appear in human fetuses during the second part of the third intrauterine month, when lymphopoiesis is already established and the cortex, and medulla, and CMJs are capable of carrying out both positive and negative selection of T cells. Differentiation mostly occurs between 6 and 9-months, but HBs are still present in adults.

The development of HBs in mice is demonstrated with a study of Vicente et al. (1996) that showed the thymic primordium of 13-day-old embryos is formed by a homogeneous population of primitive TECs, which differentiate into various TEC subtypes of both the cortex and the medulla.

At the same time, stroma-supported, keratinized, and vacuolated TECs occur in the thymic medulla.

These last two cell types differentiate subsequently into HBs and hypertrophied cells.

HBs in old thymus

Normal aged thymuses show prominent HBs with different morphology: (1) corpuscles with a characteristic concentric arrangement of keratinizing TECs and hyaline homogenate in the center; (2) giant corpuscles with flaky material, lymphocytes, and stromal cells in the middle; (3) "cellular" corpuscles with vital cells, some of which presented characteristics of active secretion; and (4) cystically degenerated corpuscles (Marinova et al. 2009). These authors showed, at light microscopical analysis, that the structural components of the majority of HBs, such as TECs, macrophages and lymphocytes showed a colocalization of insulin-like growth factor-I (IGF-I) and IGF-I receptor (IGF-IR) immunopositivity. Moreover, at the ultrastructural level, these cells had cytoplasmic localization of the IGF-I granules complexes in thymic TECs and macrophages. The granules were mainly dispersed around the Golgi complex and mitochondria. It is known that IGF-1 is a growth factor for the immune system (Kelley et al. 2007). It increases the number of lymphocytes in the thymus and spleen and enhances their function through a greater lymphocyte generation and survival. Moreover, IGF-I suppresses apoptosis in thymocytes stimulating autoimmunity (Savino et al. 1995).

Thymic vasculature

An important but less studied component of the thymic cytoarchitecture is the network of blood vessels, sometimes called the "thymic blood vessel tree" (Bryson et al. 2011). This network provides for oxygen delivery as in other organs, but the blood vessels in this organ have additional functions linked their role in thymocyte maturations. In spite of the crucial role played by blood vessels into the thymus, no more studies considered the development of these latter during ontogeny. Thus, we decided to add one paragraph for focusing on this point.

Vessels development in human prenatal thymus

The human thymus showed a vascular cytoarchitecture since the 10th week prenatally. At 12th weeks, late normoblasts and granulocytes increased around blood spaces in the interlobular septa and cortical and medullary vasculature increased. At 16 weeks, nerve bundles accompanied arteries and veins and, between the 20th and 24th weeks, radial cortical capillaries drained into capsular venules. The arterioles gave a series of radial cortical capillaries and less regular vessels to the medulla. From the 28th to the 40th week prenatally the vascular thymic supply was markedly increased and cortical capillaries may anastomosed (Ghali et al. 1980).

Yamasaki (1989) and Ghali et al. (1980) observed also that the thymus abnormalities in the arterial supply are more frequent in fetuses than in adults.

The human arterial supply and venous drainage in adult and old thymus

It is well established that the blood supply to the thymus is usually from the inferior thyroid, of which they constitute either a collateral or a terminal branch, internal thoracic, pericardiacophrenic or anterior intercostals arteries; whereas only rarely may arise from middle thyroid artery(Safieddine and Keshavjee 2011). The thymus is drained chiefly by vessels that are tributaries to the left brachiocephalic and internal thoracic veins, however, variation is fairly common (Cahill 1998).

The laterally thymic arteries, asymmetrical and variable in number, usually originate from the internal mammary artery, mainly on the right, and only occasionally from the superior phrenic artery, a branch of the internal mammary artery (Safieddine and Keshavjee 2011). The lateral thymic arteries reach the thymus by traversing the fibrous wall of the thymus sheath originating numerous fine branches (Di Marino et al. 1987). Often, the artery arising from the internal thoracic vessel enters the thymic compartment, but it is destined for the pericardium and pericardial fat (Di Marino et al. 1987).

Posterior thymic arteries are rare, but not exceptional; they are direct branches of the brachiocephalic artery and aorta, usually they form an unique branch that split into different branches at the level of both human thymic lobes before entering the capsule (Safieddine and Keshavjee 2011; Di Marino et al. 1987).

Finally, it is important to remember that have been described also some accessory thymic arteries; that arise from diverse sources: superior thyroid, subclavian and thyrocervical trunk (Di Marino et al. 1987).

Whereas, in human thymic venous drainage, it was observed that the venous system does not run parallel to thymic arterial supply and that the thymic veins are small but numerous (Safieddine and Keshavjee 2011; Di Marino et al. 1987). In particular, venous draining system is characterized by larger veins that follow the interlobular septa into the thymic capsule and small veins that leave the cortex from venous plexus of the posterior surface of the capsule. Only the veins entering the left brachiocephalic trunk are sizeable, their diameter often reaching 3 mm. These veins usually leave the medial border or posterior aspect of each of the two lobes and generally join to form a single vessel which reaches the anterior aspect of upper border of the brachiocephalic vein (Di Marino et al. 1987).

The right lobe's drainage is to the left brachiocephalic vein, the right internal jugular vein, the right jugulo-subclavian confluence, the right internal thoracic vein, the inferior thyroid vein, and the median thyroid vein (Di Marino et al. 1987). The grand veins of Keynes (thymic posterior veins) are formed by the fusion of numerous smaller veins which drain the thymus and, in turn, empty into the brachiocephalic vein justproximal to the origin of the posterior intercostals vein. Superior thymic veins drain mainly the superior part of the gland and empty into the inferior thyroid vein. There are other small veins that drain into the superior vena cava and the internal mammary vein (Safieddine and Keshavjee, 2011) (Fig. 9).

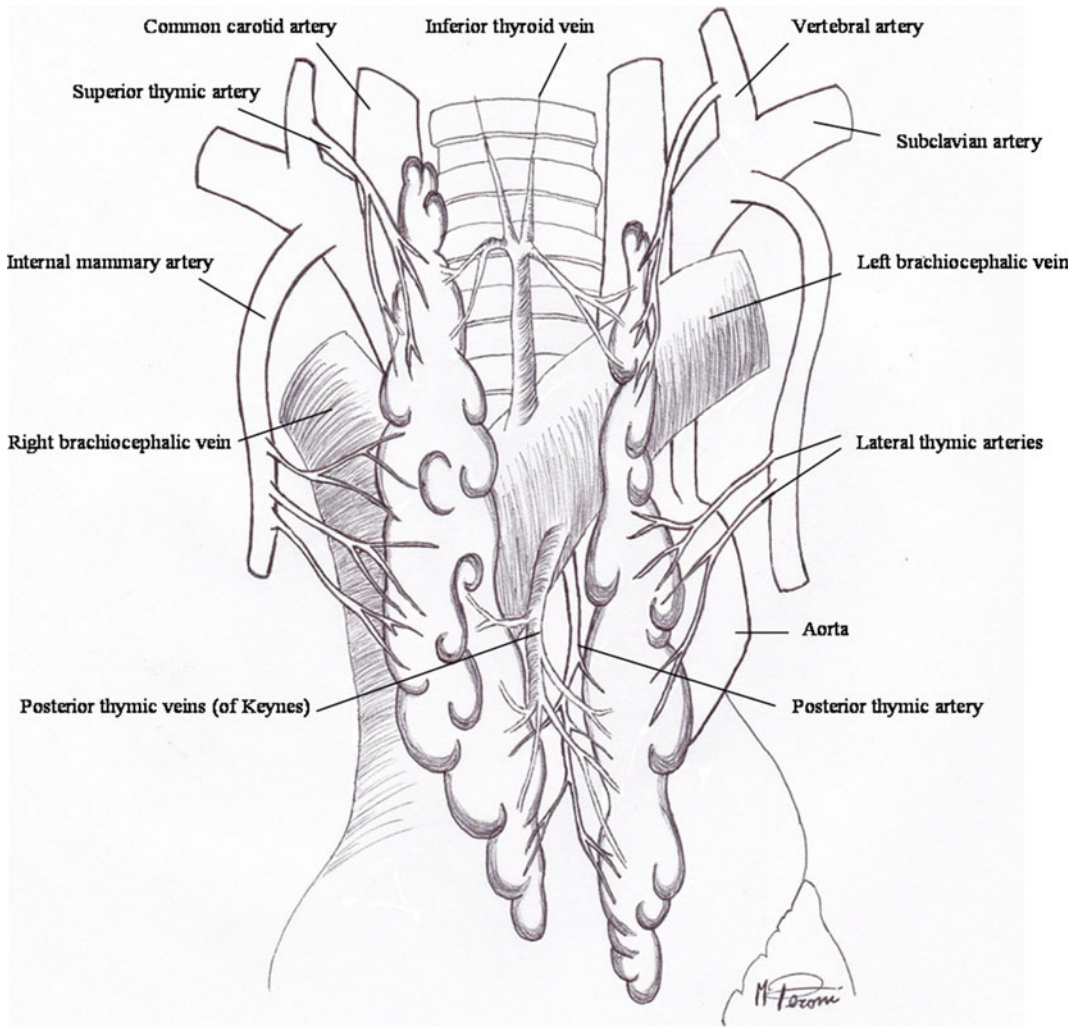

Fig. 9 Schematic representation of thymus arterial blood supply and vein drainage (modified from Safieddine and Keshavjee 2011)

It is important to remember that although exist a lot of abnormalities in the arterial thymic supply. In particular, Yamasaki (1989) investigated the arterial system of human thymus in 43 fetuses and 77 adults and observed, among the anomalous arteries, that the middle thymothyroid artery showed the highest frequency of 44.2 % in fetuses and of 27.3 % in adults. The superior thymic artery and the middle thymic artery were also abundant, being 33.7, 18.8, 32.6, 14.3 % in fetuses and adults, respectively. On the other hand, the supreme thymic artery and the thyroid ima artery arising from the internal thoracic artery were extremely rare (Yamasaki 1989).

Moreover, Cahill (1998), through a careful dissection of the neck and thorax of an 81-year-old male, cadaver revealed anomalous branch to the thymic arterial supply. In particular, the author observed that the anomalous branch to the thymus arose from the anterior surface of the common carotid artery approximately 1 cm above the bifurcation of the brachiocephalic artery. It coursed inferiorly through a plexus of inferior thyroid veins in front of the brachiocephalic artery and crossed the anterior surface of the trachea. Just superior to the left brachiocephalic vein it bifurcated into right and left branches that entered the right and left lobes of the thymus respectively. The artery was 6 cm in length before bifurcation. The thymus also received several small twigs from the pericardiacophrenic arteries and its veins drained predominately into the left brachiocephalic vein.

There is potential clinical significance in this anormalities in the field of the operating surgeon in the performance of endarterectomy or whenever clamping of the carotid artery is involved which might cause vertebrobasilar ischemia (Cahill 1998).

Moreover, Bearman et al. (1975) observed through electron microscopy analyses a characteristic vascular–parenchymal relationship. The vascular lumen is always separated from the thymic parenchyma by the endothelial cell cytoplasm, a muscular coat in arterioles and veins, the vascular basal lamina, a PVS containing collagen fibers and cells, the epithelial–reticular cell basal lamina and the epithelial–reticular cell cytoplasm. The width of this PVS is proportional to the size of the vessel it surrounds: it is wide around the vessels in the septa and at the cortical–medullary junction but narrow around capillaries and many cells are present in this space around larger vessels, but only collagen was observed around the capillaries.

Vessels development in murine natal and postnatal thymus

A kind of population of cells (neural crest-derived mesenchyme) migrate inside the thymus before ED 13.5. From their differentiations, smooth muscle cells associated with large vessels and pericytes associated with capillaries come out. In the adulthood, these cells are maintained in the parenchyma providing structural support to the vascular architecture (Foster et al. 2008; Müller et al. 2008). These endothelial precursors have been studied using 3D reconstructions of vessels. The Authors concluded in this study that different regions of the thymus have specific types of vessels: capillaries in the cortex, medium size vessels associated with medullary region, and large vessels without a precise localization (Anderson et al. 2000). The presence of medium size vessels in medulla suggested that interaction between vasculature and mTECs are responsible for organizing the medullary compartment.

During the first week of postnatal life, the neonatal thymus contains a dense, immature, and VEGF-dependent vasculature consisting of fine, branching capillaries, higher levels of CD31 endothelium, and very few pericytes. A possible role of VEGF is found inside the growing process of the vascular thymic system. In fact, this hormone is produced by thymocytes and TECs and permits the expansion of endothelial cells. The level of VEGF is high during the murine neonatal period and reaches approximately the value of zero in adulthood. Moreover, the inhibition of VEGF signaling during neonatal life induces a rapid loss of the dense capillaries in the thymus and profoundly reduces thymopoiesis.

It is possible that changes/alteration to the neonatal thymic vasculature could influence ingress of thymocyte precursors, and that permeable, VEGF-responsive vasculature may permit highly efficient migration into the neonatal thymus (Cuddihy et al. 2009).

Another marker was discovered inside TECs: Foxn1. This gene starts to be expressed around the ED 12.5 and is involved in appearance of TECs (Mori et al. 2010). The expression of Foxn1 is essential for thymus organogenesis (Nehls et al. 1994).

On ED12, the thymus anlagen are separated from the pharynx, and form an epithelial clustered mass (Itoi et al. 2001). The thymus anlagen begin to attract T cell progenitors from blood vessels adjacent to the thymus anlagen and to support T cell development in parallel

withorgan development (Itoi et al. 2001). In the thymus of postnatal mice, thymic blood vessels have roles not only in providing oxygen and metabolites but also in the immigration of T cell progenitors into the thymic epithelial region, the emigration of mature T cells to the periphery (Mori et al. 2007; Petrie 2002), and maintenance of the thymic architecture (Anderson et al. 2000).

The murine postnatal thymus contains a unique vascular cytoarchitecture, different from adult thymus

According to Cuddihy et al. (2009), the mature and hierchical architecture of the adult vasculature consists of a range of blood vessel types with larger arterioles and venules predominating and relatively little vessel branching. This is previously reported by Raviola and Karnosky (1972) in a study carried out by ultrastructural analysis. In particular, they showed that the vascular supply of the thymic lobes is unusual because of the pattern distribution of the vessels and the geometry of the vascular tree. The intralobular vessels are represented by arterioles, capillaries and venules; a clear-cut distinction between three kinds of vessels is easily visible in electron micrographs. The arterioles run at the boundary between cortex and medulla and give off capillaries, that ascend into the cortex, being joined to each other by collateral anastomoses (Fig. 10). At the periphery of the cortex, but still within the parenchyma, the capillaries form a network, as above reported, of branching and anastomosing arcades looking to

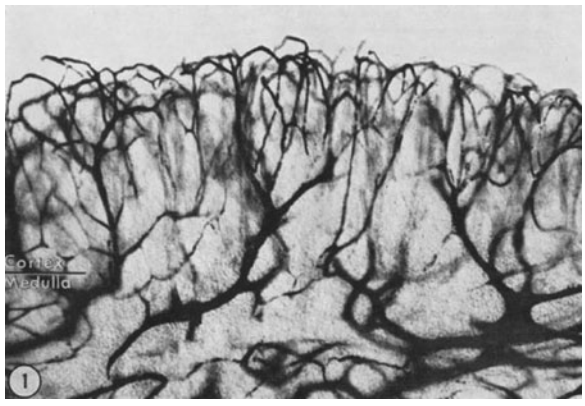

Fig. 10 Photomicrograph of a 200-μm section of mouse thymus after vascular injection with photographic emulsion. Original magnification, ×200 (modified from Raviola and Karnovsky 1972)

interior of the lobe. In the cortex, they join to form larger vessels, that are again classified as capillaries on the basis of their structures. These vessels merge into PCVs at the CMJ and in the medulla. In addition to the capillaries coming from the cortex and to the PCVs, small capillaries originating directly from the arterioles at the CMJ supply the medulla. The intralobular vessels through the parenchyma are among several types of cells being included a system of canals bounded by a continuous investment of reticular cell processes, macrophages and lymphocytes. All the vessels in the cortex are capillaries. They have endothelium, basal lamina, and a thin adventitial layer of collagen fibrils.

At the periphery of the cortex, the endothelium has fenestrae. A number of capillaries without fenestrae is more evident in the connective tissue of the capsulae. These cells are in association with heterogeneous clusters of connective tissue cells, macrophages, adipose cells and fibroblasts.

The arterioles (10–15 μm in diameter) are found in the CMJ and their lumen have a stellate shape for a highly irregular outline of the adluminal surface of the endothelium. The luminal surface of the endothelial cells is consistently provided with marginal folds near the tortuous intercellular junctions. The endothelial cells show many cytoplasmic organelles such as Golgi complex, which is very prominent. The elastic interna is thin and provided with wide fenestrations. A single layer of smooth muscle cells, in arterioles of smaller diameter is present; the adventitia is very thin and consists of bundles of collagen fibrils interspersed with elastic fibers.

PCVs consist as large vessels located in the CMJ and medulla; their walls present many lymphocytes. Their diameter ranges from 10 to 50 μm; their walls consist of endothelium, basal lamina, and connective tissue adventitia. The endothelium is thick and, the endothelial cells of the venules have the same architecture of capillary endothelium, but the Golgi complex is more developed. Micropinocytotic vesicles are highly variable in number from cell to cell.

Lepique et al. (2003) characterized the expression of the main mediators of leukocyte extravasation in adult mice thymus and observed that that PCVs in the postnatal thymus of mice express a number of adhesion molecules related to rolling, tethering and firm adhesion, suggesting a potential role for these in the leukocyte extravasation in secondary lymphoid organs or in inflamed tissue.

Among the studied mediators, in our opinion, it is important to describe both vascular adhesion protein-1 (VAP-1) and MECA-79.

VAP-1 is a member of a newly described class of endothelial adhesion molecules that has a known role in leukocyte extravasation, and it is upregulated by inflammatory cytokines (Kaitaniemi et al. 2013). The analysis of VAP-1 in adult mice thymus showed that its expression was restricted to a very few venules found near the CMJ and that it was not homogenous, showing a higher expression towards the cortical aspect of the vessel, whereas in deep cortical venules the distribution was homogenous around the perimeter. Moreover, the authors observed that the venules within the medullary compartment did not express VAP-1 (Lepique et al. 2003).

MECA-79 is another endothelial adhesion molecules which mediate the initial capture and rolling of lymphocytes along the vessel wall. The analysis of the expression of MECA-79 showed that it was higher in thymi from mice at 5 and 9 weeks of age and weaker in tissue from animals at 4 and 7 weeks of age. In particular, it was found to be expressed mainly on PCVs deep in the cortex or in the cortico-medullary junction (Lepique et al. 2003).

Kuwabara et al. (2002) investigated, for the first time, MECA-79 expression comparing human normal neonatal thymuses, thymomas, thymic carcinomas, and thymic lymphoid hyperplasias and observed that in normal thymuses, peripheral lymph node address in expression was found in the endothelium of corticomedullary and medullary vessels surrounded by PVS. In a subtype of thymomas and thymic lymphoid hyperplasias, peripheral lymph node addressin was detected in the vessels with PVS, at the medullary differentiation areas and in paralymphoid follicles, respectively. However, in a subtype of thymomas and thymic carcinomas, MECA-79 was expressed at vessel level of the remnants of pre-existing thymic tissue and they were absent at neoplastic level.

It is important to underline VAP-1 appears to have significant potential as a candidate regulating the specificity of progenitor homing to the post-natal thymus via the blood and expecially that the fluctuations in MECA-79 expression are sufficient to regulate the periodicity of the thymus recruitment process. However, since adhesion molecules expressed by thymic endothelial cells are also found on blood vessels in a variety of other tissues, it is likely that additional signals are required to impart specificity to the thymic homing process (Lepique et al. 2003).

The venule walls are often infiltrated with large numbers of migrating lymphocytes and their architecture can be so remarkably distorted that the vessel is not so easily distinguished from the surrounding parenchyma. In contrast, a dense highly branched network of capillaries and small vessels was seen throughout the postnatal thymus. This situation is less evident in the adult thymus. In particular, Cuddihy et al. (2009) showed a greater vascular density in postnatal thymus compared with adult thymus by endothelial cell marker (CD31) and immunofluorescence techniques. Analysis of smooth muscle actin staining revealed that pericytes were almost absent in postnatal thymus, which is consistent with the presence of immature vasculature.

In conclusion, the murine thymus is served by thymic arteries which split into arterioles as they enter the organ via an incomplete connective-tissue septum. The vascular tree of adult thymus follows a specific organization: arterioles enter the thymic parenchyma at the CMJ and form a good number of capillaries that go into the cortex. Inside this area, all of these vessels create anastomoses. After this, orientation capillaries curve down to the medulla and they constitute venules.

Inside the parenchyma, both in young and adult animals, there are four patterns of vascular supply: (1) in most cases one artery enters and one vein exits at the hilus on the dorsolateral surface of the thymus; (2) one artery enters and two veins exit at the hilus; (3) one artery enters and vein exits anteriorly; and (4) one artery enters and two veins exit anteriorly. The pattern is not always the same for both lobes of a single animal (Kato and Schoefl 1989). These authors showed also the vasculature pattern in hydrocortisone-injected animals that induced rapid involution already after half a day of its injection. In involuted thymuses the cortical capillaries were noticeably shortened and were less dense than in normal thymus. A prominent feature of the hydrocortisone-treated thymuses was the general tortuosity of the blood vessels. This contrasted with the retention of the normal vascular pattern in physiologically involuted thymuses of old animals in which there appeared to be a more generalized diminution of the vascular bed (Fig. 11).

PCVs, localized in CMJ and especially in medulla, as above reported, are analogous to the high endothelial venules of lymphoid tissues where large numbers of lymphocytes traverse the vascular wall. However, in

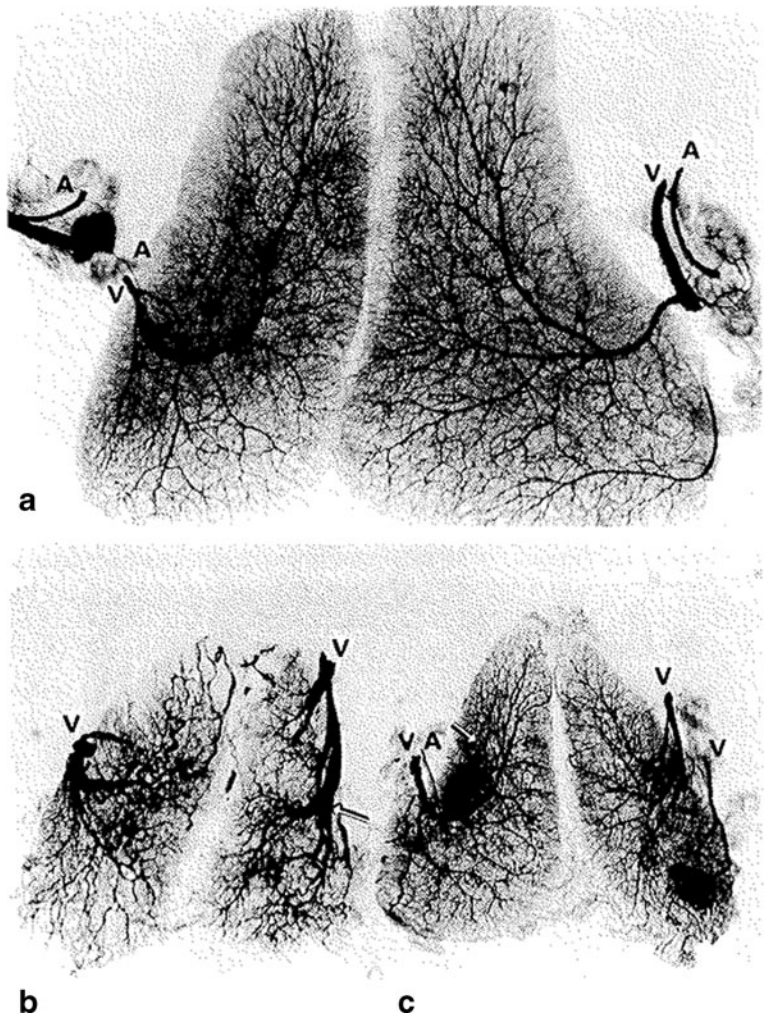

Fig. 11 Identification of the vascular supply of the normal and involuted mouse thymus. Ventral surface of thymus lobes of: an untreated thymus in which the blood vessels have been injected with Microfil and a silicon rubber compound (**a**); similarly injected thymus 3 days after hydrocortisone treatment (**b**) and untreated thymus (**c**). *A* thymic artery, *V* thymic vein. Original magnification, ×13 (modified from Kato and Schoefl 1989)

the thymus, they were thin-walled and lined by attenuated endothelial cells. The distribution and structure of these venules in the involuted thymus were similar to those observed in the normal thymus. However, in the involuted thymus, the endothelium was more heavily infiltrated with lymphocytes and even appeared to contain phagocytosed erythrocytes (Kato and Schoefl 1989). PCVs are surrounded by PVS. Endothelium of these vessels is thin, but sometimes in humans and mice it is possible to find high endothelial venules. The internal surface of PVS is formed by the basal lamina of PCVs and the other side of that space is covered by TECs. The cytoplasmic processes build up sheets, forming the PVS. Where PVS is reduced in width, there is collagen matrix and a small rim of cytoplasm of pericytes between two layers of basal laminae (Kato 1997). The basal lamina has many gaps through which cells can pass. The PVS is not a continuous longitudinal channel but is crossed by epithelial trabeculae. In addition, there were interruptions or gaps in the outer epithelial sheet through which lymphocytes appeared to migrate. Similar gaps in the outer wall of the PVS have been described not only in the thymus of rodent, like of mice (Ito and Hoshino 1996), rats (Kotani et al. 1967; Pereira and Clermont 1971), and guinea pigs (Kato and Schoefl 1987), but also in humans (Bearman et al. 1975).

In the periphery of the cortex, TECs extend protrusions to separate thymic parenchyma and connective tissue or other structures such as blood vessels and capsules (Ushiki 1986).

Inside the medulla there are arterioles and venules of intermediate diameter. It is important to notice that in the subcapsular, there is no venous drainage.

Thymus through radiological techniques and its evaluation

The thymus gland is composed of two lobes and it is normally located in the superior and anterium mediastinum, just behind the manubrium of the sternum (Wang et al. 2011) (Fig. 12a). In rare cases, this gland may found at an ectopic site, at any level of the pathway of normal thymic descent, from the angle of the mandible to the superior mediastinum. Thymus shape and size have a pretty large range of variability and change throughout the life of the subject, being large in infants and pre-adolescent period and gradually coalescing and being substituted by fat in the early adulthood (Wang et al. 2011; Chowhan et al. 2010; Herman and Siegel 2009; Mizia-Malarz et al. 2009; Prasad et al. 2006) (Fig. 12b). On ultrasonographic (US), computed tomographic (CT), and magnetic resonance (MR) imaging techniques, the thymus is represented by a triangular bilobed gland with homogeneous parenchyma voided of mass effect on the surrounding structures (Fig. 12c).

Given the variability in shape and size, the imaging of the thymus is quite complex especially using conventional radiography. On the anteroposterior view of a chest radiograph the thymus is barely discernible from the cardiac silhouette (Nasseri and Eftekhari 2010) (Fig. 12d). The borders of the gland are smooth and are seen in radiograph of infants and children younger than four years: when the "wave sign" and the "sail sign" are present they may help the physician to identify the gland. The "wave sign" is the image determined by the impression of the anterior reflection of the ribs on the thymus; the sail sign is the results of the anatomic relation between the pulmonary minor scissor and the right lobe of the thymus.

US technique is very reliable to assess the thymus parenchyma. The echogenicity of the thymus varies with the age (Nasseri and Eftekhari 2010). The thymus is similar or slightly less homogeneous when compared with the echogenicity of the spleen and the liver in the infants. It is gradually spotted by hyperechoic dots (fatty replacement) as subject ages until it shows the starry sky pattern created by a multitude of fatty

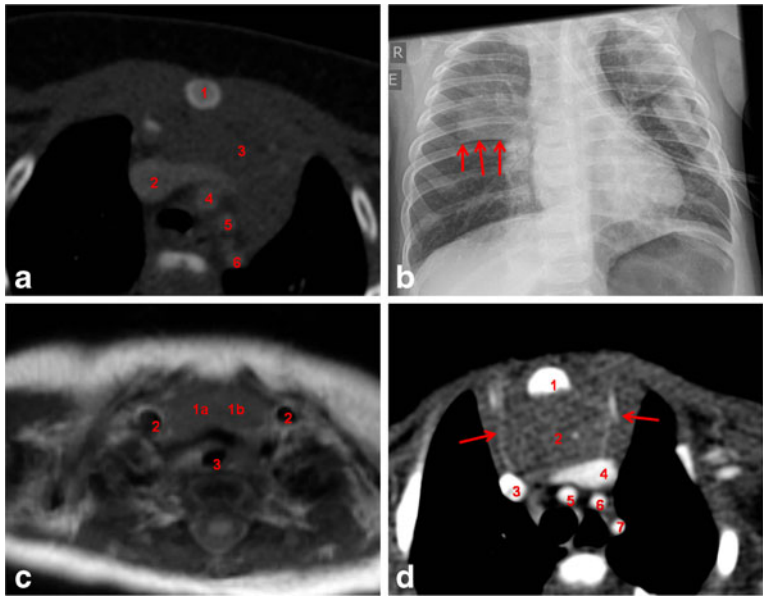

Fig. 12 Thymus evaluation through radiological techniques. **a** CT image without contrast, cut through the upper anterior mediastinum of a 5-month-old boy. *1* Sternum, *2* superior vena cava, *3* thymus, *4* innominate artery, *5* left carotid artery, *6* left subclavian artery. **b** Frontal chest radiograph of a 7-month-old boy showing with *arrows* the thymic sail sign, a triangular, slightly convex right lobe of the thymus with a sharply demarcated base caused by the minor fissure; it is seen in approximately 5 % of children. **c** MRI image of a 10-month-old female. T2-weighted fast-spin echo, cut through the superior aspect of the thymus gland. *1a* Right thymus lobe and *1b* left thymus lobe; *2* jugular vein and *3* trachea. **d** CT image with contrast, cut through the upper anterior mediastinum of a 7-week-old female. *1* Sternum, *2* thymus, *3* superior vena cava, *4* brachiocephalic trunk, *5* innominate artery, *6* left carotid artery, *7* left subclavian artery. *Arrows* indicate the internal thoracic vessels

hyperechoic foci. US technique not only demonstrates the parenchymal structure of the thymus, but may also identify thymic consistency when helping in differential diagnosis with tumors and diffuse infiltrative processes. When the US exam of a normal thymus is performed, the gland shows change in shape during patient motions (neck movements, breathing, and cardiac pulsations); the gland is more rigid and less prone to change in presence of tumors.

CT is useful to assess the main important anatomic characteristics: location, size, shape and relation with the surrounding structures. Again in the infants, thymus occupies a large portion of the superior anterior mediastinum and has a quadrilateral appearance, gradually becoming atrophic with a triangular shape in the early adulthood (Nasseri and Eftekhari 2010). On CT images, conventionally, the thymus is measured perpendicular to its long axis and the normal maximum diameter is 18 mm before 20 years and 13 mm in adults (Takahashi and Al-Janabi 2010). The main advantage of the CT is the possibility to assess the possible involvement of surrounding structures and the possible spread of diseases through the adjacent or distant organs (Gawande et al. 2012); the use of high-resolution multiplanar reformation images offers a better assessment of tiny details, often essential for a correct diagnosis. The intravenous injection of contrast media (iodium) may help in the differentiation tissues and in determining the extent of an eventual pathological process.

When compared with CT, MR technique has pros and cons. The main disadvantages of MR are the longer scanning length (30–45 min) compared with the 15–30 s of CT; the required patient collaboration is a minimal risk of nephrogenic systemic fibrosis in patients with renal failure (Ackman and Wu 2011). On the other hand, MR overcomes the radiation exposure risk of the CT and offers a better evaluation of the soft tissue located within the mediastinum compared. Although CT is superior to MR in the assessing of the calcification within the gland, MR can evaluate the structure of the gland evaluating septa, capsule (Benveniste et al. 2011), and gland composition; in particular, the use of the chemical shift artifact allows the quantification of fat and water within the tissue and may give useful information for diagnosing (Inaoka et al. 2007; Nasseri and Eftekhari 2010). To suppress breathing and cardiac pulsation artifacts, specific techniques such as cardiac or respiratory gating or breath-hold examination with ultra-short sequences are used. The electrocardiography (ECG)-triggered sequences can provide very high quality images and details regarding anatomic structures. For the respiratory artifacts, a tool for monitoring the movement of the diaphragm to obtain selected images is available (Takahashi and Al-Janabi 2010). To achieve a successful breath-holding examination, the acquisition time has to be limited as much as possible; this is possibly limiting the MR to the area of interest by only focusing on a few structures (Ackman and Wu 2011). Injection of contrast media (gadolinium) can improve the spatial resolution and increase the signal to noise ratio; in images with contrast, media is mandatory for the use of suppression of the fat signal to achieve sufficient anatomic detail to distinguish lymph nodes and eventual masses from the surrounding fat (Kato et al. 2012). Use of dynamic contrast-enhanced magnetic resonance imaging is a must in the assessment of vascularity and steady-state gradient echo imaging can demonstrate shape and patency of the great vessels (Takahashi and Al-Janabi 2010). A dedicated phased-array surface coil has to be used (Inaoka et al. 2007). DCE- patterns for different thymus lesions have been described recently in the literature. For instance, Sakai et al. (2002) demonstrated that the mean time to peak enhancement was very short for thymomas when compared with thymic lymphomas and carcinoma (Sakai et al. 2002). The morphological sequence as T1- and T2-weighted spin-echo (SE) sequences for thymus can describe the aging of the thymus: in infant and young children the gland is homogeneous with intermediate signal, lower than the mediastinal fat but higher than the muscle; in persons older than 25 years, the thymus is no longer seen as a proper structure but it is seen as islands of T1 and T2 intermediate signal tissue within fat tissue; over the age of 40 years, it has the same signal of the fat (Takahashi and Al-Janabi 2010). Dynamic studies with diffusion-weighted imaging may help in obtaining specific information such as the apparent diffusion coefficient. This coefficient estimates the diffusion space of water protons in the extracellular matrix and has been used as a predictor of malignancy (Liu et al. 2006).

Autonomic innervation of lymphoid organs an neuroimmune modulation

An increasing body of evidence indicated the occurrence of functional interconnection between the

immune and nervous systems, although data available on the mechanisms of this bi-directional cross-talking are frequently incomplete and not always focused on their relevance for neuroimmune modulation.

Primary (bone marrow and thymus) and secondary (spleen and lymphonodes) lymphoid organs are supplied with an autonomic (mainly sympathetic) efferent innervation and with an afferent sensory innervation (Mignini et al. 2003).

Different fibers and neurotransmitter of thymic innervation

Mignini et al. (2003) showed that the thymus of rodents is supplied by sympathetic noradrenergic innervation endings at the level of thymic capsule, subcapsular region, and connective septa.

Thymic sympathetic innervation originates from postganglionic cell bodies located in the superior cervical and stellate ganglia of the sympathetic chain (Tollefson and Bulloch 1990). In postnatal and adult rodents, postganglionic noradrenergic fibres enter the thymus associated with large blood vessels as dense nerve plexuses, and travel across the capsulae and interlobular septa or follow vasculature until it reaches the cortex. Nerve fibres originating from these plexuses end in the cortex and supply vasculature to parenchymal regions of both outer and deep cortex. In the subcapsular area around the vasculature, noradrenergic nerve profiles are predominantly limited to the cortex with a slightly higher density near the CMJ. Thus, noradrenergic fibers of septal origin run in venous sinuses from where they reach thymic cortex and medulla (Cavallotti et al. 1999). In the capsule and interlobular septa, noradrenergic nerve fibers course adjacent to mast cells (Bellinger et al. 1992). In the outer cortex, where thymocytes are located and develop, sympathetic profiles are in close relationship with thymocytes. In the deep cortex and medulla, sympathetic profiles are adjacent to TECs.

Parasympathetic acetylcholine esterase (AChE)-positive nerve fibers reach the thymus via vagus, recurrent laryngeal and phrenic nerves (Bulloch and Pomerantz 1984). Thymic vagal innervation that was investigated in the rat using electrophysiological techniques consists primarily of non-myelinated C-fibers. Right and left lobes of the thymus are supplied bilaterally by fibers from cervical vagus (Niijima 1995). AChE-positive nerve fibres pass from the capsular region to the cortex and reach the medulla running in association with blood vessels (Mitchell et al. 1997). AChE-positive cells were also found in rat thymic medulla (Al-Shawaf et al. 1991). Developmental studies have shown that AChE-positive nerve fibres appear in the capsule, interlobular septa and in subcapsular and cortico-medullary areas of rat thymus, around the 18th day of fetal life. The density of these profiles increases during development. Specific AChE activity in rat thymus is detectable around the 19th day of gestation. A significant increase in the activity of this enzyme is detectable around the third day after birth and remains approximately at the same level up to the 90th day of age. This suggests that the thymus receives parasympathetic innervation relatively early in ontogeny and that these nerves are potentially involved in the regulation of the organ activity, acting on thymocytes and/or modulating activity of TECs.

ChAT immunohistochemistry was shown to label fine perivascular nerve fibres in thymic parenchyma (Fatani et al. 1986). The thymic rudiment is not innervated during early ontogenetic development and the first ChAT-positive nerve profiles are detectable around the 17/18th days of gestation.

Moreover, noradrenergic sympathetic innervation, neuropeptide Y (NPY)-containing nerve fibres extend from subcapsular and septal plexuses, into superficial and deep cortical regions in association with vasculature or as individual fibers, arborizing between thymocytes and associated cells. The densest innervation occurs at the CMJ.

The distribution of vasoactive intestinal peptide (VIP)-positive fibres is similar to that of NPY-containing nerve fibers, although they are expressed with a lower density (Mitchell et al. 1997). Some VIP immunoreactive fibres have a non-perivascular localization at the CMJ or run adjacent to perivascular mast cells and macrophages.

Sparse cells positive for calcitonin gene-related peptide (CGRP) were seen during the late embryonic period at the CMJ and in the medulla. The number of these cells increases in adulthood and is reduced in the thymus of aged mice (Bulloch et al. 1994). Thymic and lymph node lymphocytes synthesize CGRP that probably acts as a paracrine mediator in immune cells (Xing et al. 1998).

Neurotransmitter systems involved in the innervation of thymus are summarized in Table 6.

Table 6 Neurotransmitter systems in thymus of rats and mice

Neurotrasmitter	Animal species	Targets
Norepinephrine	Mouse	Thymocytes
	Rat	TECs
Dopamine	Mouse	Thymocytes
Acetylcholine	Rat	Thymocytes, TECs, and noradrenergic nerve terminals
Neuropeptide Y	Mouse	Thymocytes
Vasoactive intestinal peptide	Rat	Thymocytes
	Mouse	Thymocytes
Calcitonin gene-related peptide	Rat	Thymocytes
	Mouse	Thymocytes
Substance P	Mouse	Thymocytes

Innervation in postnatal, adult, and old thymus

According to Bellinger et al. (1988), thymus shows sympathetic postganglionic noradrenergic (NA) innervation in postnatal and adult rodents and it is able to maintain this innervation during involution too. Moreover, the authors suggest that these fibers provide a NA-enriched microenvironment for interaction with adrenergic receptors on thymocytes.

Another studies (Madden et al. 1998) indicated that, in the postnatal rodents, thymic NA innervation was found in the capsule and subcapsular cortex, where stem cells arrive from the bone marrow. In the medulla and cortex, where T cells undergo differentiation and maturation, NA innervation was associated with septa and blood vessels.

In old rodents, NA nerve fibers associated with septa and blood vessels increased in density. In very old rodents, NA innervation surrounding vessels and septa was dense and tortuous. Numerous NA nerve fibers coursed throughout the parenchyma, with no association with the vasculature or septa.

Thymic norepinephrine (NE) concentration increased with age, but when the reduced thymic weight was taken into account, total thymic NE was not significantly altered. This finding suggests that thymic NA innervation is maintained as the thymus involutes with age (Table 7). The thymocytes in the involuting thymus reside among a higher density of NA nerve fibers, suggesting that they are exposed to higher concentrations of NE as the animal ages.

Thus, these findings suggest that the thymus is able to maintain NA innervation during aging and this is important for interaction of adrenergic receptors of thymocytes.

Thymic involution

The involution starts in the periphery of lobules with the invasion of adipose tissue. PVS between blood vessels and the surrounding cells in the thymus increases and it is infiltrated with adipose tissue, lymphoid cells and phagocytized erythrocytes. This is correlated with a reduction in the export of new T cells into the periphery. In addition, this feature is explained with a decrease of neural crest-derived cells, that support vascular tree (Cavallotti et al. 2008).

Age-related thymic involution is often described as being sex hormone dependent: both androgens and estrogens, male and female sex hormones respectively, have been directly associated with thymic involution (Brelińska 2003). Moreover, it is known that sex hormone ablation by chemical or surgical castration reverses age-related thymic involution and restores thymic function with a decrease in the rate of thymocyte apoptosis and increased proliferation of both T cells and TECs. TEC expression of androgen/estrogen receptors is required for thymic involution and normal thymic development so the effect of sex hormones on thymic involution is thought to be mediated by TECs (Heng et al. 2005; Hince et al. 2008). Despite this evidence that sex hormones are potent mediators of thymic involution, they are not the primary mediators of age-related involution. In fact, while sex hormone ablation reverses age-related thymic involution it also boosts the function of a nonaged thymus, suggesting a generic suppressive effect of sex hormones rather than an age-dependent effect (Papadopoulou et al. 2012), even, progressive age-related thymic involution is not

Table 7 Norepinephrine levels in thymus of aged rodents (modified from Madden et al. 1998)

Age	Norepinephrine (per mg wet weight)	Norepinephrine (per thymus)
Young	100±6	100±6
Adult	382±58	155±18
Very old	1,722±568	153±20

mirrored by progressive increases in sex hormone levels (Morley 2003). Moreover, age-related thymic involution appears to be initiated before puberty (Shanley et al. 2009) and the boost to thymic cellularity that occurs due to sex hormone ablation is transient and, if performed in young, does not prevent age-related decline (Dooley and Liston 2012; Min et al. 2006).

Human thymic involution

The acceleration of thymic decline after puberty is more pronounced in males compared with in females, suggesting that increasing levels of sex hormones contribute to the involution process. Among these hormones, androgens in particular exert considerable influence on the size and "composition" of the thymus (Chen et al. 2010). Lymphoid component in PVS undergoes a process of reduction in 20- to 25-year-old humans and at the same time PVS left by thymocytes is populated by adipocytes which concur to enlarge this area, enlarging also PVS (Haynes et al. 2000).

Analyzing the thymus during its development in humans/mice lifespan, it is important to observe the morphological view of the organ and at the same time the biological markers and their levels. Just through analysis of both aspects we can see the deeply and remarkable mutation inside the structure and the cells of thymus.

TECs produce many cytokines that undergo a modification during lifespan such as IL-2, IL-9, IL-10, IL-13, and IL-14: their levels fell down in old specimens. Despite that IL-7, IL-15, and G-CSF levels have not changed (Haynes et al. 2000). Conversely, it is observed a rising levels of leukemia inhibitory factory (LIF), stem cell factor, M-CSF, and oncostatin M just because they are well spread by adipocytes as well TECs (Sempowski et al. 2000).

CXCL12 is involved in the processes of proliferation and survival of human lymphoid precursor and belongs to the CXC class of chemokine involved inside many biological activities. IL-7 performs the same activity of CXCL12 on these cells, providing the upregulation of expression of CXCR4 on $CD34^+$ lymphoid progenitors. Simultaneously, IL-7 stimulates CXCL12 production by TECs. At the end CXCL12 downregulates the IL-7 production by TECs. In addition, DCs express CXCR4 and produce CXCL12. In old human thymus, there are no important changes in the expression of CXCL12 and CXCR4 in comparison with young thymus. However, CXCR4 undergoes a significant reduction in $CD34^+$ thymic lymphoid progenitors (Hernández-López et al. 2010). Other studies are necessary to well understand the role of CXCL12 in the survival of $CD34^+$ cells. It is important to underline that in this process IL-7 is reduced as it is shown in a study of Aspinall and Andrew (2000).

Murine thymic involution

A significant reduction of mice TECs was shown with pan-keratin antibody with wide spectrum and expression of MHC II. After 18 months of age, there are widespread areas displaying no staining. This reflects the loss of TECs, which is connected to deficit of maturation of T cells.

Aminopeptidase A, cortex-specific keratin subunits recognized with the C11 mAb and CD205 are used to identify alteration in cortex area, revealing modification in the cTEC network. All of them undergo a process of reduction with age, especially in 24-month-old mice. Whereas medullary markers are TEC adhesion molecule (Ep-CAM), K5 and UEA-1 and they stain the tissue lesser and lesser in increasing age (Aw et al. 2009). Besides, the markers that decrease are those that increase: Notch1 and Delta. In particular, Notch1 is increased in cTECs, while it does not show change in mTECs. However, Delta marker is equally increased in both TEC subpopulations. Furthermore, Notch1 is involved in T cell development and its activation does not allow differentiation of DP into SP thymocytes, noting the close link between TEC and T cell (Laws and Osborne 2004).

Many markers of increased senescence can be used on cells to establish the DNA damage that reflects the arrest of replication. Phosphorylated histone H2AX and tumour suppressor p53 binding protein 1 (53BP1) show the damaged DNA, staining especially the older thymus of 12/18-month-old mice. In addition, β-galactosidase activity reflects the pattern of aged cell: no β-galactosidase activity is present at 1-month-old mice thymus, but after 6 months the activity starts to appear, becoming prominent in 12/18-month-old mice (Aw et al. 2008).

TECs are reduced approximately one third in old mice. The same reduction is shown dealing with the proliferative cell staining with Ki67; 3-fold more is the

level of apoptotic TECs in aged mice thymus (Gui et al. 2007).

Kang et al. (2008) discovered an androgen receptor (AR) RWD domain containing 1 (Rwdd1) that is markedly decreased in aged and oxidatively stressed mice on thymocytes and TECs. AR is connected with the thymus involution. When testosterone binds, AR inhibits thymocyte development.

All strategies, including gonadectomy, to overcome the deleterious effects of thymic involution merely result in a transient increase in thymus size but do not prevent its eventual decline (Holländer et al. 2010; Taub et al. 2010; Griffith et al. 2012). These observations suggest that age-related decline is an intrinsic property of thymopoiesis. The pool of TEC progenitors is established during embryogenesis and, due to limited niche space, comprises a fixed number of such cells (Jenkinson et al. 2008), in addition, the depletion of TECs during early embryogenesis is not compensated during later stages of development, suggesting that the bipotent TEC progenitor lacks appreciable self-renewing properties (Rode and Boehm 2012.

Exogenous administration of androgens in adult rodents results in an altered cell trafficking, reduced thymocyte proliferation and an increase in thymocyte apoptosis (Olsen et al. 1998). Conversely, removal of androgen by castration results in thymic enlargement and increased thymopoiesis (Sutherland et al. 2005). Despite the clinical importance, the exact mechanisms by which thymic homeostasis is regulated by androgens remain incompletely understood (Chen et al., 2010).

The removal of testosterone by castration has been found to lead to a proliferative response of both cortical and medullary TECs, which may contribute to the trophic effects on the thymus. Clinically, a trophic effect on T cells has been demonstrated by chemical castration in patients with prostate cancer and by growth hormone treatment of adult patients suffering from growth hormone deficiency (Chen et al. 2010; Morrhaye et al. 2009).

Moreover, castrazion enhances immune reconstitution in young, adult, middle-aged, and aged male mice (18–24 months) in several immunocompromised models observing an increase in proliferation and cellularity of early thymocyte subsets, the atrophic thymus resembles that of a young thymus (Goldberg et al. 2005; Sutherland et al. 2005).

Whereas, ovariectomy of female mice and rats has also been shown to cause enlargement of the thymus, however to a lesser degree than that seen with castration in males (Utsuyama and Hirokawa 1989). The hormonal response to ovariectomy is more complex than castration causing a decrease in estrogens, prolactin, progesterone and dehydroepiandrosterone, which also have immunomodulatory effects (Deitch et al. 2006). In fact, ovariectomy is strongly age dependent: in young mice there is a profound increase in thymic size, as for males, but there is little effect on female mice 6–12 months of age. This is possibly due to the contribution of adrenal and fat metabolism derived estrogen-indeed the serum of the ovariectomised older mice still contained levels of estrogen comparable to sham-ovariectomised control mice (Hince et al. 2008). It has been long recognised that there is a profound impact of sex steroids on the immune system in general shown that the thymus returns to age-matched controls by 20 months post-ovariectomy in female mice presumably due to the gradual return of circulating estrogens (Zhao et al. 2005) or the potential reduction in supply of thymus progenitors in particular. In this context, the effects of sex steroids-androgens and estrogens, are complex and wide reaching.

Adrenalectomy of female and male mice results in thymic hypertrophy involving also a loss of glucocorticoids (GCs) (Forsberg 2000). Although, there is an increase in all thymocyte subsets (Safadi et al. 2000). While castration and ovariectomy appear to be long-term in respect to enhanced immune reconstitution, in fact thymic size is evident for at least a year. It is unknown how this may translate in the human setting (Hince et al. 2008).

In the neonatal period, estrogen at least appears to be essential for normal immune development, while post-pubertal increases in sex steroid levels suppress TESCs and TEPCs and downstream T and B cell differentiation and maturation, associated with a profound reduction in thymic output and an eventual increased risk of opportunistic infections. During periods of severe immunosuppression best typified by chemotherapy and radiotherapy, both surgical and chemical ablation of sex steroids improves immune recovery of the thymus and bone marrow and subsequently T and B lymphoid compartments. Thus, temporary sex steroid ablation has the potential to become an important tool for improving immune recovery following severe immunodepletion (Hince et al. 2008).

RhoB belongs to the Ras superfamily of GTP-binding proteins and it is important in biological

function of the cell. Its deficiency in thymic medullary epithelium is connected to a premature organ atrophy. Bravo-Nuevo et al. (2011) found that just mice at 5-weeks of age there is a reduction in thymus weight and cellularity. RhoB control development and maintenance of thymus, inhibiting the effect of TGF-β. In fact, the expression of TGF-β receptor type II is increased in RhoB-null mice, showing a major effect of TGF-β on mTECs. In particular an higher level of TGF-β1 is directly involved in thymic atrophy or, in indirectly way, by the upregulation of suppressive cytokines like IL-6 and LIF.

Thymus, brain, testes-associated gene (Tbata) regulates TEC function and thymus size. In a study of 2010 by Flomerfelt et al. (2010) aged Tbata-deficient mice show larger and more dividing TECs. In normal mice increasing Tbeta level is connected with decreasing thymus size. Tbata rises up till 4 month and reaches a steady state after that point: stromal cells do not proliferate and they undergo a decline.

Qiao et al. in 2008 found an increasing production of GCs in TECs between 4 and 22 weeks mice that has a negative effect on the development on thymocytes and providing apoptosis of that cells. There are many enzymes involved in GC synthesis and they have different proportions in young and old mice. Steroidogenic acute regulatory protein-related lipid transfer (StAR), cytochrome (CYP) 11A1, and CYP11B1 are increased in thymocytes of old mice, while StAR and CYP11A1 are decreased in thymic stromal tissue (TST). CYP11B1 remains constant. 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) enzyme, which converts inactive GC into active form, has the same level in T cell population and TST in 4 weeks mice. This expression is decreased in TST and increased in T cells of 14 weeks mice. 11β-HSD2 enzyme, which converts active GC into inactive form, is increased in thymocytes of old mice. 11β-HSD2 expression is 50-fold less than 11β-HSD1. 3β-HSD, CYP21A, and CYP17 do not have differences between postnatal and old mice.

Thymocytes

CD3 is involved in the T cell receptor (TCR) signaling and the overall percentage of $CD3^+$ thymocytes does not show alteration, even if in 1-month-old mice there is a little decrease. Associated with TCR there is CD5 that negatively controls the signaling: it has an increment with age. Another molecule CD24, marker of the most immature thymocytes, decreases its level in 18 months old mice. An important rising up of CD69 is detected in thymocytes between 6 and 18 months of age: CD69 is upregulated in the final step of maturation of T cells (Aw et al. 2010).

T cell population has an increasing production of microRNAs (3-fold or more). This alteration is connected with the cellular protein profile and consequent alteration in protein generation of that cell. That increasing is studied on triple negative thymocyte ($CD3^-$, $CD4^-$, and $CD8^-$) which has just entered the thymic parenchyma and can be one of the causes of the age-related block in thymopoiesis (Virts and Thoman 2010).

Analyzing a peripheral proliferation marker of T cells (signal joint TCR rearrangement excision circles) a lower level of it in old specimens (more than 75-year-old people) is noticed. At the same time, the higher expression of CD57 and shortened telomeres are significant to underline the aging of thymocytes, in fact these two markers are replicative senescence-related markers (Mitchell et al. 2010; Ferrando-Martínez et al. 2011).

Macrophages and DCs

The secretion of IL-1, IL-6, and TNF by macrophages falls down with age, while prostaglandin2 production rises up. These observations are present just in mice and not in elderly humans. The flow cytometric analysis shows a similar expression pattern of CD45RO, CD54, CD13, CD80, CD86, and CD40 in postnatal and elderly people, as well as chemokine receptors CXCR1, CXCR3, CXCR4, and CCR2. The same levels of MHC class II molecules on macrophages in age mice and on monocytes of old people have been detected.

In old DC class II molecules, CD54 and CD86 are reduced, while GM-CSF and IL-4 have no age differences (Varas et al. 2003).

Murine adipocyte transformation

In the aged thymic parenchyma there is an increasing number of adipocytes and this phenomena was studied in mice by de Mello Coelho et al. (2009). They demonstrate the enhancement of expression of CCR5 and its ligands (CCL3, CCL4, and CCL5) in the old

thymus. Beside this, there is a raising of adipocyte differentiation markers (peroxisome proliferator-activated receptor γ2 (PPARγ2) and adipocyte fatty acid-binding protein) and improvement of migration ability when they treat cells with CCR5 ligands that reflects the abundant presence of fat-storing cells inside aged mice thymus.

A study by Kvell et al. (2010) showed the important role of Wnt4, produced after the upregulation of Foxn1, in maintaining the features of TECs. In old mice, the expression of Wnt4 is decreased as Foxn1 and is accompanied by an increase of lamina-associated polypeptides 2α, PPARγ, and adipose differentiation-related protein. These three markers are connected with the differentiation of fibroblast in to pre-adipocyte.

Foxn1 rules medullary and cortical TEC differentiation during ontogeny of murine thymus. Producing a novel allele of the gene, Chen et al. (2009) demonstrated that the downregulation of Foxn1 below 50 % caused the thymic early degeneration, underlining the need of wild-type genes to maintain the postnatal thymus. Foxn1 is also important in the development of vascularization of thymus. Endothelial cells $CD31^+$ start to be observed at mice ED 12–13 and at the same time VEGF and platelet-derived growth factor receptor markers stain the outer mesenchymal layer (Mori et al. 2010).

Axin level is elevated in aged periods and increased in particular within thymic stromal cells and medullary TECs (not in cortical TECs) and it is supposed to be connected to the adipogenesis of the murine thymus (Yang et al. 2009a, b, c).

Human adipocyte transformation

In all vertebrates studied to date, aging of the thymus is accelerated compared with aging of many other organs. Thymic aging is characterized by dramatic reduction in thymocyte numbers and marked perturbations in the thymic stromal cell microenvironment. In contrast to a young thymus where thymocytes are the major contributors to the thymic microenvironment, adipocytes constitute the bulk of aged thymic cellular space (Flores et al. 1999; Yang et al. 2009a, b, c). This age involution is shown by a decrease in the overall weight of the organ, associated lymphoid tissue atrophy and replacement by mature adipose tissue. For this reason, all the studies carried out on adult thymus involution have been focused primarily on the immunological aspect.

By 50 years of age, approximately 80 % of thymic stromal space is dysfunctional and composed of adipose tissue. Age-related thymic involution is associated with reduced immune surveillance, increased risk and severity of emerging infections, cancers, vaccination failures, and delayed T cell reconstitution in patients undergoing hematopoietic stem cell transplantations (Lynch et al. 2009; Holland and van den Brink 2009).

Previous studies have demonstrated that the adipocytes and thymocytes can come in close cell–cell contact in the human thymus during aging (Cavallotti et al. 2008). It was also established that adipocytes are not inert cells and, depending upon their location, can secrete distinct cytokines and hormones that influence the local and systemic environment and immune function (Tilg and Moschen 2006). On the contrary, several studies over the past few years support the hypothesis that adipocytes differentiate through specific adipogenic mechanisms and this process may compromise hematopoietic and thymic function (Dixit 2012; Yang et al. 2009a, b, c; Youm et al. 2010).

Tinahones et al. (2009) have observed that thymus fat produces a variety of angiogenic factors such as angiopoietin, VEGF and VEGF receptors (VEGF-R1 and VEGF-R2) and so they found, accordingly to Salas et al. (2009), that adult thymus fat is an interesting source of angiogenic factors that might affect thymic function/s and ongoing adipogenesis within the thymus. Tinahones et al. (2009) concluded that the thymus replaces its immune function by another which is that of an ordinary white adipose tissue. Moreover, it is evident that thymus fat presents a similar cellular profile as that observed in other white adipose tissues such as subcutaneous adipose tissue. This cellular variety seems to be responsible for the observed differences in the expression of a variety of genes, known to be relevant in the regulation of angiogenesis and adipogenesis in other white adipose tissues (Tinahones et al. 2009).

Thymus and diseases

Aging is responsible for several morphological and functional changes in the lymphoid organs and in particular in the thymus. This produces an increase in the sensitivity to infectious diseases, cancer, and it is linked in part to diabetes, obesity, and hypertension.

Thus, we report some studies linked to these latter diseases.

Diabetes

It is demonstrated that insulin plays a crucial role as a thymic growth factor. Insulin, growth hormone, and induction of insulin growth factor-1 (IGF-1) by GH provide the positive effect on the replication and development of lymphocyte inside the parenchyma (Brimnes et al. 2002). Insulin is almost always found in the medulla.

In type 1 diabetes (T1DM), we attribute immunological alterations with a decrease of naïve T cells, a lower level of TRECs, telomeric shortening and decrement of IL-7. In this chronic autoimmune condition, β-cells of pancreas do not produce insulin because of the destructive effect of autoreactive T cells against pancreatic islets of Langerhans (Csorba et al. 2010). Hofer et al. in 2009 verified the effect of long-term therapy with insulin in children and adolescents with T1DM. The result of using this drug is a normalized situation in the thymic function, in opposition with what we say above. Therefore insulin performed a protective function in T1DM.

Considering both type of diabetes and the elevated concentration of seric glucose, Gruia et al. (2011) observe an increasing thymocyte apoptosis and a reduction of thymic weight during a process of hyperglycemia induced with streptozotocin in a mouse-model. In the same study it is seen as an elevation of free fatty acids inside macrophages, mostly arachidonic acid.

Nonobese diabetic mice (NOD) spontaneously develop autoimmune disease that closely resembles human T1DM and provides an excellent animal model for studying differentiation of pathogenic Th1 effectors (Zhang et al. 1994).

Patients with T1DM have been shown to have decreased levels of $CD4^+$ $CD25^+$ T cells compared with normal controls and patients with T2DM. NOD mice also have an apparent deficiency in the number of $CD4^+$ $CD25^+$ T cells, seen primarily in the thymus and spleen and the deficiency of these cells is associated with an inability to maintain peripheral tolerance to self-antigen. Neonatal anti-TNF injection results in an increase of peripheral $CD4^+$ $CD25^+$ T cells, so the number and function of $CD4^+$ $CD25^+$ regulatory T cell is influenced by the inflammatory cytokine milieu and the direct or indirect effects on regulatory T cells may be a principal mechanism by which TNF regulates autoimmunity in the neonatal NOD mouse (Wu et al. 2002).

Savino et al. (1991) observed, in both males and females NOD mice, a strong decrease in the cell numbers of discrete medullary TEC subsets, namely, those respectively defined by the expression of cytokeratins 3/10 and cytokeratin 19. In addition, there was also a significant early decrease production of thymulin, thymic anti-infiammatory hormon, in females, as compared with males. As regards the extracellular matrix compartment, the most striking alteration was the presence of abnormally enlarged PVSs, increasing in size with age. In these structures large amounts of T cells and, to a lesser extent, B-cells were consistently encountered. In addition to B-cells, the NOD mouse thymus showed on both TEC and extracellular matrix the presence of deposits of immunoglobulins. These data clearly demonstrate that the NOD mouse thymus undergoes a variety of microenvironmental changes, whose particular role in the pathophysiology of the disease is yet to be demonstrated (Savino et al. 1991).

Obesity

Obesity and its attendant metabolic disorders represent one of the great public health challenges of our time. Obesity is a medical condition in which excess body fat conducts to a multisystem disorder associated with a reduction of lifespan and quality of life and an increased probability to have acute and chronic infections (Haslam and James 2005). This status is also partnered with type 2 diabetes (T2D), cardiovascular diseases and carcinogenesis (Flegal et al. 2007).

Despite this a caloric restriction without malnutrition promotes an elongated last of life and prevents thymocytes depletion in the thymus, typical of immunosenescence during aging (Barger et al. 2003; Yang et al. 2009a, b, c). In addition nosocomial infection and postoperative complications are more abundant in obese people (Flegal et al. 2006).

The workgroup of Hyun won in 2012 observed in 13 month old mice of obese condition provided by a marked caloric diet many alterations. First of all, an increased perithymic adipose tissue is shown in these mice and thymic parenchyma gradually disappear in that perithymic layer. Inside the organ the total number of thymocytes is reduced with a general lowering of

medulla and cortex cell population: SP $CD4^+$ and $CD8^+$ cells and DP $CD4^+$ $CD8^+$ are reduced, also with an accentuation of thymocyte apoptosis. Also the CMJ tends to disappearing. The logical consequence is a decrease in peripheral T cells studied with the count of TRECs and an increased presence of memory T cells.

A new finding by Trottier et al. (2012) suggested that in a rodent model of diet-induced obesity the obesity significantly altered hematopoietic and lymphopoietic functions in the bone marrow and thymus. The data show that mice fed a high fat diet (45 % mixed fat) and so developing obesity substantially increased the number of nucleated cells in the marrow and thymus for the 180-day-period studied. This finding alone is in marked contrast to the effects of aging, inflammatory bowel disease, stress, etc., that cause depletions in the primary immune tissues as above reported. These Authors suggests a complex interplay among the metabolic factors, cytokines, and adipokines that accompany obesity, and therefore, the end result is a promotion of lymphopoiesis and myelopoiesis.

Mice lacking functional leptin (*ob*/*ob*) or leptin receptors (*db*/*db*) are common animal models for respectively obesity and diabetes and so for metabolic syndrome. *Ob*/*ob* and *db*/*db* mice exhibit increased susceptibility to infection and decreased Th1-type responses. Likewise, the reduction in plasma leptin levels observed for humans and rodents subjected to caloric restriction correlates with clinically significant T cell deficiency, impaired adaptive immune function and markedly increased susceptibility to infection (Ahima et al. 1996; Neumann et al. 2004). It is important to underline that the deficient adaptive immunity observed in the setting of deficient leptin function is thymic atrophy. Specifically, *ob*/*ob* and *db*/*db* mice exhibit a marked reduction in thymocyte cellularity, particularly of the $CD4^+$ $CD8^+$ double-positive subset (Fujita et al. 2002; Hick et al. 2006). Reduced thymocyte frequency correlates with markedly enhanced thymocyte apoptosis in *ob*/*ob* mice. *ob*/*ob* mice also have decreased thymopoiesis compared with age-matched controls, with thymic atrophy that is reversed by exogenous leptin administration (Matarese et al. 2005). Whereas, the density of mast cells in skeletal muscle, liver, spleen, and thymus was not affected by leptin deficiency in *ob*/*ob* mice (Altintas et al. 2012).

Moreover, also *db*/*db* mice showed a decrease in thymic weight and a marked reduction of DNA synthesis in the thymuses. These data suggest that the altered metabolic status of the diabetic host influences immune function *in vivo* possibly due to abnormal function of lymphocyte subpopulations (Fernandes et al. 1978)

Similarly, in humans and rodents with low leptin levels, the cellularity of the thymus is dramatically reduced principally due to significant loss of cortical thymocytes (Savino 2002; Trotter-Mayo and Roberts 2008).

In addition, it has been appreciated that thymus contain deposits of adipose tissue capable of producing leptin (Pond 2000; Sempowski et al. 2000).

Actually, it is clear that leptin is needed for proper immune function and inflammatory, therefore, leptin plays a pleiotropic role in cytokine modulation and is an important link between the neuroendocrine and immunologic systems so leptin administration may be an important therapeutic strategy to enhance T cell reconstitution in the human clinical settings of stress and leptin depletion (Hick et al. 2006).

Not only in old obese mice is it possible to discover the lowering of thymic output but also in middle-aged humans with high body max index (BMI>30). In humans, the correlation between obesity, TRECs number and T2D do not produce a higher reduction in T cell production. In fact, an obese subject with or without T2D has the same TREC count. Thus, age-related thymic involution is known to be associated with a significant decrease in TECs, increase in adipogenic fibroblasts, and deterioration of thymic stromal microenvironment (Yang et al. 2009a, b, c).

Concluding remarks

In this review, we intended to highlight that the involution of the thymus has profound consequences on the function of the immune system. Thus, it plays a central role in the development of immunosenescence. Several pathologic contexts (associated with thymic ablation or hypoplasia) offer the opportunity to directly assess the importance of the thymus, and how the loss of its function may affect the immune system in humans (Appay et al. 2010). This is a tough task, but certainly other future studies will represent new advances in the appreciation of the role of the thymus in humans and animals. This perspective will be extremely important for understanding of the development of immunosenescence. Thus, we reported the

knowledge about the morphology, function and radiological evaluation of the thymus during the lifespan of rodents and humans, where the data are available.

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