

Thymus microenvironment in human primary immunodeficiency diseases

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Purpose of review

Severe combined immunodeficiencies represent a heterogeneous group of genetic disorders affecting genes of both early and late steps in lymphocytes development, a process tightly controlled by thymic epithelial cells. Detailed analysis of thymic morphology aids to the assessment of the severity of the immune disorder and may be critical to the understanding of the role of the genetic defects in the pathophysiology of these diseases. In this review, we highlight recent advancements in the characterization of the thymic microenvironment in primary immunodeficiencies.

Recent findings

Crosstalk between thymocytes and thymic epithelial cells is essential to preserve thymic architecture and function, and therefore to promote T-cell maturation and development of self-tolerance. Early severe defects in T-cell development result in profound abnormalities of thymic epithelial cells differentiation with loss of AIRE expression and severe reduction of thymic dendritic and T-regulatory cells. Differently, later defects in T-cell development that are permissive for normal thymocytes development allow cortico-medullary differentiation with partially preserved AIRE expression and dendritic/T-regulatory cells distribution. Hypomorphic mutations in the same genes partially permissive to T-cell development may result in a more complex phenotype with immunodysreactivity and peculiar thymic alterations.

Summary

Although the molecular and genetic bases of primary immunodeficiencies directly aid to both diagnosis and management of the patients, the detailed analysis of thymic morphology critically contributes to unveil the pathophysiology of these diseases.

Keywords

autoimmunity, primary immunodeficiency, thymus microenvironment

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Introduction

A large variety of molecular mechanisms may account for primary immunodeficiency diseases (PIDs) in humans, including genetic defects affecting both early and late steps in T-cell development [1–3]. Typically, these defects result in selective severe depletion of circulating T lymphocytes, with variable involvement of B and/or natural killer (NK) lymphocytes as well [2,4]. Despite their genetic heterogeneity, PIDs share a common clinical phenotype consisting of early-onset, severe respiratory infections, protracted diarrhea and failure to thrive. Null mutations in genes involved in early T-cell development characterize severe combined immunodeficiencies (SCIDs) with virtual lack of circulating T lymphocytes [5,6]. Later defects in T-cell development compromise developmental stages beyond the CD4⁺CD8⁺ (double-

positive) stage in thymocyte development and affect production of single-positive CD4⁺ cells or of single-positive CD8⁺ lymphocytes [3,7,8]. These deficiencies differ from SCID for the presence of a residual number of circulating T lymphocytes and are commonly referred to as combined immunodeficiency (CID) [3]. In contrast, hypomorphic mutations of the same genes allow a partial, although abnormal, T-cell development and result in a more complex phenotype leading to the unique association of immunodeficiency and autoimmunity [9,10,11[•]].

The thymic microenvironment is the main structure in which lymphoid cells mature and differentiate upon interaction with thymic stromal cells [12^{••},13,14^{••}], but at the same time, signals delivered by the developing lymphocytes are crucial to induce maturation of thymic epithelial cells (TECs) and to support maintenance of

thymic architecture and function [15–18]. However, the organization and maturation of thymic microenvironment in human PIDs remain poorly defined [19,20]. There are evidences in the literature that early and late defects in T-cell development differently affect the cellular organization of the thymic microenvironment [21,22[•]]. We have previously shown that early severe lymphoid defect in human primary immunodeficiencies results in disrupted thymic architecture with abnormal maturation of TECs and depletion of naturally T-regulatory cells (nTregs) and thymic medullary dendritic cells [22[•]]. Moreover, we have shown that thymus from patients with PIDs carrying hypomorphic mutations of early genes partially permissive to T-cell development had severe abnormalities of TECs maturation. In particular, we have observed a marked reduced expression of autoimmune regulator (AIRE), a key factor driving the expression of tissue-restricted antigens (TRAs) [23–25], providing clues on the pathogenesis of the peculiar association with autoimmune manifestations [22[•],26,27].

In this review, we provide a schematic overview of how different molecular defects in T-cell development affect thymic environmental organization and maturation, and impact on the pathogenesis of PIDs.

Normal thymus: from morphology to function

The thymus is a highly specialized primary lymphoid organ composed of functionally interdependent lymphoid and stromal components that create a peculiar microenvironment in which lymphoid progenitors undergo differentiation/maturation and acquire a functional T-cell repertoire [13,28[•]]. Understanding the morphological and functional features of the normal thymus aid to investigate its alteration in PIDs, in which variability of the molecular defect in the T-cell development reflects different thymic morphological perturbations.

Normal thymic architecture

Histologically, the thymus shows a lobular structure, each lobule consisting of two different regions: the cortex, characterized by densely packed small immature lymphocytes within a delicate sparse epithelial network, and the medulla, less densely cellular and containing T-cells at different stages of maturation and prominent epithelial cells (Fig. 1, panel a) [29]. Other cell types (B-cells, plasma cells, mast cells and eosinophils) represent a minor fraction, with the exception of macrophages, predominantly distributed in the cortex, and dendritic cells, almost exclusively present in the medulla and cortico-medullary junction (CMJ) [28[•]]. A peculiar feature of the medullary region is the presence of Hassall's bodies, round structures formed by highly keratinized fully mature epithelial cells, marked by strong positivity for staining with *Ulex europaeus* agglutinin 1

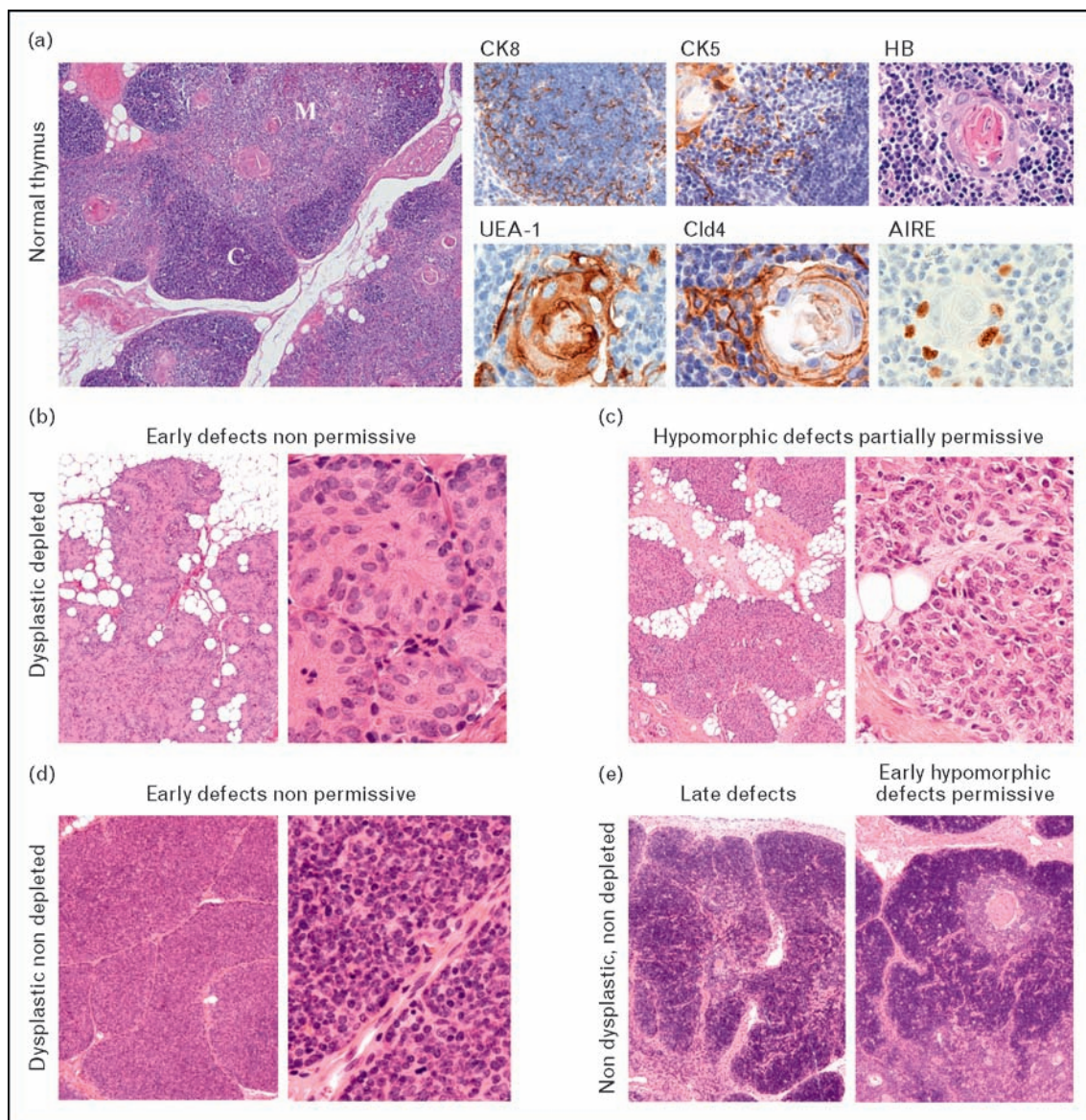
(UEA-1) (Fig. 1, panel a). Cortico-medullary differentiation (CMD), expressed as the ratio between cortex and medulla, is considered one of the main parameters for the histopathological evaluation of thymic maturation [19]. Morphometric analysis of thymic biopsies from human young adults shows a normal cortical to medulla ratio of 1.2–1.3 (P.L. Poliani, personal communication). Differential expression of cytokeratin-5 (CK5) and cytokeratin-8 (CK8) identifies different subsets of TECs, with TEC progenitors being mostly CK5⁺CK8⁺ and mature cortical TECs (cTECs) and medullary TECs (mTECs) being mostly CK8⁺CK5[−] and CK8[−]CK5⁺, respectively [30,31].

Intrathymic T-cell development

The immature lymphocytes enter the thymus at the CMJ and undergo maturation, as they pass through the cortex to the medulla and finally leave the thymus as mature peripheral T-cells [7,14^{••}]. Early thymic progenitors (ETPs) are lineage negative expressing Sca1, c-kit, TdT and L-selectin (Lin[−] c-kit^{high} L-selectin⁺ TdT⁺) with some of them retaining B lineage potential (Flt/Flk-2⁺) [32]. Interactions with the thymic stroma promote the initial phase of T-cell differentiation marked by the expression of CD7, CD1a and CD2 [7,16]. During cortical migration, developing T-cells (thymocytes) proliferate and differentiate from an immature double-negative CD4[−]CD8[−] to intermediate double-positive CD4⁺CD8⁺ phenotype. In the cortex, thymocytes that rearrange the T-cell receptor (TCR) acquire T-cell competence and are positively selected [33]. The successful engagement of TCR in the context of major histocompatibility complex class I (MHC-I) molecules leads to differentiation into single-positive CD4[−]CD8⁺ T-cells, whereas engagement of TCR with the MHC class II molecules leads to differentiation into single-positive CD4⁺CD8[−] T-cells [7,34]. Single-positive T-cells are located predominantly in the medulla, in which they closely interact with TECs.

The thymic microenvironment

Crosstalk between thymocytes and TECs is essential to preserve thymic architecture and function, and it is necessary to promote and support T-cell development and migration of thymocytes through the thymic cortex to the medulla, in which deletional and nondeletional mechanisms of central tolerance take place [12^{••},18,35,36]. Recent data have shown that positively selected thymocytes foster TECs maturation and thymic medulla formation by signals mediated by receptor activator of nuclear factor (NF)- κ B (RANK) and CD40 [37,38]. Deletion of negatively selected autoreactive thymocytes in the medulla is based on the recognition of self-antigens that are presented by both mature mTECs and thymic dendritic cell [33]. In this process, a key role is played by the transcription factor AIRE, which drives expression of TRAs by a subset of mature mTEC with marked positivity for claudin-4 (Cld4) and UEA-1 [39,40]. Recent

Figure 1 Morphology of normal thymus (panel a) and thymuses in primary immunodeficiency diseases associated with different T-cell defects and maturation

PID thymi show various pathological patterns, defined (see Table 1 for details) as dysplastic/depleted (panels b and c), dysplastic/nondepleted (panel d) and nondysplastic/nondepleted (panel e). These patterns are related to the general architecture of the gland, its CMD differentiation, HB formation and the lymphocyte content. In panel a, the immunophenotype of normal thymus is also shown, with expression of CK8 by cTECs and CD5 by mTECs, in association with UEA-1, Cld4 and AIRE (all hematoxylin and eosin stain, except immunostains in panel a as indicated). AIRE, autoimmune regulator; C, cortex; CK5, cytokeratin-5; CK8, cytokeratin-8; Cld-4, claudin-4; CMD, cortico-medullary differentiation; cTECs, cortical thymic epithelial cells; HBs, Hassall's bodies; mTECs, medullary thymic epithelial cells; PID, primary immunodeficiency disease; UEA-1, Ulex europaeus agglutinin 1.

data suggest that in humans, thymic stromal lymphopoietin (TSLP) secreted by Hassall's bodies epithelium activates thymic dendritic cell [41–43] that diverts autoreactive T-cells into Foxp3^+ Tregs [44]. Tregs represent an important player in the development of peripheral tolerance [34,45]. In this way, crosstalk between Hassall's bodies, mTECs, thymic medullary dendritic cells and thymocytes all contribute to the T-cell development and establishment of self-tolerance.

Morphological and functional features of the thymus in primary immunodeficiencies

As thymic microenvironment represents a complex integrated network of different cell types contributing to T-cell development, molecular defects affecting thymocytes result or are associated with morphological alterations of the thymic gland [3,46]. The spectrum of morphological changes in the thymus associated with

primary and secondary immunodeficiencies has been described as dysplasia or atrophy, respectively [19,47]. The major features of dysplasia consist of severe depletion of thymocytes, lack of epithelial cell maturation with absence of Hassall's corpuscles and fatty infiltration; they have been associated with severe inherited diseases, most commonly primary SCIDs. Different degrees of morphological anomalies within dysplasia have been considered to reflect the severity of the disorder and not to be associated with a specific disease [19,47]. In contrast, thymic atrophy is characterized by regressive changes developing after birth and consists of shrinkage of the gland with collapsed and disorganized epithelial cell network; lymphocytes are depleted, but inflammatory cells, particularly plasma cells, are found. Thymic atrophy is associated with several conditions such as acquired immunodeficiencies, severe malnutrition, graft-versus-host disease, cytotoxic drugs and chronic steroid treatment [47]. Only recently, thymus changes in PIDs have been more precisely related to the molecular defect [20,22[•],48], and the following paragraphs will discuss the main findings of these studies.

Early and severe defects in T-cell development

Thymic dysplasia is the hallmark of severe PIDs carrying a genetic defect that severely affect early stages in T-cell development. Hale *et al.* [21] had previously shown that thymic microenvironment differentiates abnormally in thymus from patients with X-linked SCID. This study demonstrated that in addition to T-cells, both thymic dendritic cells and TECs development is affected in SCID. Interestingly, thymic biopsies from these patients were markedly hypoplastic with profound alteration of their architecture, loss of CMD and absence of Hassall's bodies. The authors concluded that whether the abnormal development of dendritic cells and TECs results directly from the genetic defect or secondarily from lack of normal crosstalk between developing thymocytes and maturing TECs remains to be clarified.

Thymic biopsies from patients carrying null mutations in the common γ -chain [interleukin 2 receptor gamma (*IL2RG*)] and in adenylate kinase 2 (*AK2*) genes [22[•], 49–51] show severe thymic dysplasia with loss of CMD, reminiscent of primitive embryonal thymic epithelium and severe depletion of thymocytes (Fig. 1, panel b).

Interestingly, thymic biopsies from PID patients whose gene defects [null mutations in *CD3D* and recombination activating gene 2 (*RAG2*) genes] occur in a later developmental step as compared with the *IL2RG* gene and abrogate signalling through the pre-TCR show a different pattern of thymic morphology consisting of a pronounced lobular architecture with thin collagen septa; in addition, the lobules are rather cellular and contain thymocytes

along with a disorganized epithelial cell network; also in these cases, no CMD is identified [19,22[•]] (Fig. 1, panel d).

Independently from the morphological pattern, all these conditions associated with early and severe defects in T-cell development show a similar immunophenotype of thymic epithelium, mostly composed of immature CK5⁺CK8⁺ cells, with predominance of CK8 expression and lack of Cld4⁺UEA-1⁺ and AIRE-positive mTECs. Furthermore, these thymi reveal severe depletion of myeloid and plasmacytoid medullary thymic dendritic cells, as shown by staining with antibodies against S-100 protein, CD11c and CD303/Blood DC Antigen (BDCA)-2 [22[•]]; similar findings have been reported in patients with X-linked SCID [21].

Hypomorphic defects permissive or partially permissive to T-cell development

Hypomorphic mutations in genes involved in early or late T-cell development differentially affect the thymic microenvironment, depending on the degree of the genetic defect that could be permissive or only partially permissive to T-cell development. Omenn syndrome, commonly due to hypomorphic mutations in the *RAG1* or *RAG2* genes [52–54], represents the major example of such conditions, although mutations in the interleukin-7 receptor (*IL7R*), *IL2RG*, DNA cross-link repair 1C (*DCLRE1C*) and RNA component of mitochondrial RNA processing endoribonuclease (*RMRP*) genes [9] have been also associated with the Omenn syndrome phenotype. The clinical manifestations of Omenn syndrome (erythrodermia, lymphadenopathy, hepatosplenomegaly and severe diarrhea) reflect T-cell-mediated infiltration and damage of target tissues by autoreactive oligoclonal T-cells, accounting for unique association of immune deficiency and autoimmunity [26,27,55,56]. Furthermore, hypomorphic defects in these genes may also cause leaky SCID, in which residual T-cell development may associate with the presence of activated T-cell in the periphery, without the marked T-cell infiltrates and tissue damage typical of Omenn syndrome [3,9]. The mechanisms underlying such phenotypic variability remain poorly defined.

Thymuses from patients with Omenn syndrome (associated with hypomorphic mutations of *RAG2* [26,27] and *RMRP* [57] genes) show lobules composed of epithelial cells devoid of CMD differentiation and with markedly reduced lymphoid cell component (although less marked than in the previous category) (Fig. 1, panel c); peripheral condensation of epithelial cells may result in a pseudo-CMD, but immunostain confirms the lack of epithelial cell compartmentalization, and TECs are largely CK5⁺CK8⁺ [22[•]]. In keeping with the lack of medullary differentiation, Omenn syndrome patients thymi are

Table 1 Morphological and immunophenotypic findings in thymi from primary immunodeficiency diseases

Thymic pattern	Genetic defect in T-cell development	Thymic architecture	Lymphoid component	Epithelial component
Dysplastic, depleted	Early severe defects nonpermissive to T-cell development abrogating signalling from ETP to pro-T-cell progenitors, e.g.: null mutations in the <i>IL2RG</i> and <i>AK2</i> genes Early hypomorphic defects nonpermissive or partially permissive to T-cell development, e.g.: hypomorphic mutations in the <i>RAG2</i> and <i>RMRP</i> genes (Omenn) Early defects nonpermissive to T-cell development abrogating signalling from pro-T to pre-T progenitors, e.g.: null mutation in the <i>RAG2</i> and <i>CD3D</i> ^a	Epithelial nests and cords, with gland-like structures, fatty tissue infiltration; no CMD [22 [•]] Lobules separated by thin collagenous septa, composed of immature lymphoid cells and stromal cells; no CMD Normal architecture with low M/C; CMD present Normal architecture; CMD present	Severe depletion with near absence of T- cells; isolated CD2 ⁺ cells can be found [21,50,51] Severe-to-moderate depletion with presence of CD2 and CD3 cells [22 [•] ,26,27] Nondepleted; numerous immature DN thymocytes [19]	Diffuse network of immature CK5 ⁺ CK8 ⁺ TECs; no HBs; no Cld-4 and AIRE expression
Dysplastic, nondepleted	Late defects or early hypomorphic defects permissive to T-cell development e.g. null mutation in the <i>ZAP70</i> and <i>MHC-II</i> genes; hypomorphic <i>R222C</i> mutation in the <i>IL2RG</i> gene	Normal architecture; CMD present	Mild medulla depletion [19] Normal	Mature CK5 ⁺ CK8 ⁺ cTEC and CK5 ⁺ CK8 ⁺ mTECs; presence of HBs, Cld-4 and AIRE

AIRE, autoimmune regulator; AK2, adenylate kinase 2; CK5, cytokeratin-5; CK8, cytokeratin-8; Cld-4, claudin-4; CMD, cortico-medullary differentiation; cTEC, cortical thymic epithelial cell; DN, double negative; ETP, early thymic progenitor; HBs, Hassall's bodies; *IL2RG*, interleukin 2 receptor gamma; M/C, medulla-to-cortex ratio; *MHC-II*, major histocompatibility complex class II; mTECs, medullary thymic epithelial cells; *RAG2*, recombination activating gene 2; *RMRP*, RNA component of mitochondrial RNA processing endoribonuclease; TECs, thymic epithelial cells; *ZAP70*, zeta chain-associated protein kinase 70 kDa.
^a Data are referred to deficiency of the delta chain of CD3. It should be noted that in humans, deficiency of different CD3 chains might result in diseases with different severity [4,19].

depleted of Cld4⁺UEA-1⁺ mTECs and do not express AIRE.

Interestingly, one patient carrying hypomorphic *IL2RG* R222C [58] mutation permissive to T-cell development has shown a normal thymus morphology and immunophenotype, including TECs maturation and compartmentalization, formation of Hassall's bodies and Cld4 and UEA-1 and AIRE expression (Fig. 1, panel e).

Maturation of thymic dendritic cells is also severely affected in Omenn syndrome patients but preserved in patients carrying permissive hypomorphic *IL2RG* R222C mutation [22[•]]. In analogy, Foxp3⁺ cells are found only in the thymus from patients carrying hypomorphic *IL2RG* R222C mutation, whereas they are very few in Omenn syndrome patients [22[•],27,55].

Late defects in T-cell development

Mutations that are permissive to T-cell development allow TECs differentiation and maturation, resulting in a normal morphology, cytoarchitecture and phenotype, including the expression of AIRE and a normal content of FoxP3⁺ cells (Fig. 1, panel e). However, in a patient with zeta chain-associated protein kinase 70 kDa (*ZAP70*) [19,59,60] deficiency, the medullary-to-cortex ratio is reduced, possibly reflecting impaired thymocyte signalling (P.L. Poliani, unpublished communication).

Table 1 summarizes the morphological and immunophenotypic findings in thymi from different PIDs.

Conclusion

The large majority of PIDs whose genetic defect has been identified can be easily diagnosed on the basis of clinical features, lymphocyte phenotyping and molecular studies. Thymic biopsy represents an invasive procedure, and it is, therefore, unnecessary as a diagnostic procedure. However, in selected cases, investigating the morphological/functional changes of the thymus can be crucial for defining type and severity of the immunodeficiency, which in turn may affect management and treatment decisions. Moreover, investigation on thymic morphology and immunophenotype can contribute to the understanding of the pathophysiology of PIDs and to get clues on the function of the normal immune system in humans.

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Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 574–578).

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