



Tight Interdigitating Developmental Processes within the Thymus; Lessons from Primary Immunodeficiency with Autoimmunity

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Abstract

The underlying cause of the enigmatic coexistence of immunodeficiency and autoimmune disorders in patients with primary immunodeficiency such as Omenn syndrome is largely due to the inefficient negative selection within thymus where T cells develop. Recent advances in molecular biology and animal models answered one of the key questions on the relationship between the partially impaired T cell development at early stages and the presence of autoreactive T cells in periphery. T cell development and thymic organogenesis are tightly coupled to each other. The maturing T cells induce thymic epithelial cell development and AIRE (*Autoimmune regulator*) expression in thymic medullary epithelial cells. Without thymic medullary epithelial cells and AIRE protein in the patients with primary immunodeficiency, a few autoreactive T cells survive through the complete thymic selection processes and expand to form oligoclonal T cell pools in the periphery. The induction of thymic medullary epithelial cell development may be considered as an alternative therapeutic approach in addition to the current standard therapy for primary immunodeficiency - stem cell transplantation.

Keywords: Primary Immunodeficiency, Autoimmunity, T cell development, Thymic organogenesis.

Box 1. Primary Immunodeficiency (PID)

PID is a heterogeneous group of diseases with more than 180 disease types. The number is still growing with advances in diagnostic technology and increases in early diagnosis. It is characterized by genetic defects in various components of the innate and adaptive immune system, such as neutrophils, macrophages, dendritic cells, complement proteins, natural killer cells, and T and B lymphocytes. The study of these diseases has provided essential insights into the functioning of the immune system [1, 2]. PID is distinct from Secondary Immunodeficiency Disease originated from infections or treatment of immunomodulatory drugs [3]. The complexity and diversity of the PID has prompted the need for their classification for the diagnosis and treatment (Table 1). DiGeorge syndrome, Wiskott-Aldrich syndrome, Omenn syndrome, Severely Combined ImmunoDeficiency (SCID) belongs to Cellular or Combined Defects type in Table 1. They are well known PIDs in which various genetic defects were found, resulting in abnormalities in DNA repair and/or thymus development [2, 4].

Table 1 Type of primary immunodeficiency diseases

Type of Primary Immunodeficiency Diseases	Disease	ICD 9* code
Antibody Production Defects	X-linked Agammaglobulinemia (Bruton's)	279.04
	Common Variable Immunodeficiency (CVID)	279.06
	X-linked or Autosomal Hyper IgM Syndrome	279.05
	Selective IgA Deficiency	279.01
Cellular or Combined Defects	Severe Combined Immunodeficiency	279.2
	DiGeorge Syndrome	279.11
	Ataxia Telangiectasia	334.8
	Wiskott-Aldrich Syndrome	279.12
Phagocytic Cell Immune Defects	Leukocyte Adhesion Defect	288.9
	Chronic Granulomatous Disease	288.1
	Chediak Higashi Syndrome	288.2
	Cyclic Neutropenia Kostman Diseases	288.0
Complement Defects	C1 Esterase Inhibitor Deficiency	277.6
	Complement Component Deficiency	279.8
	* ICD9: International Classification of Disease 9 th version	

The prevalence of PID

There appear to be regional differences in the prevalence of PID. For example, incidence in Sweden is 0.35 per 100,000 persons, while in southern Australia a prevalence of 12.4 per

100,000 persons is observed, and it rises to 1 in 1,200 persons in United States. Immunoglobulin deficiency accounts for 70-80% of all PID [1, 5, 6]. For the remaining 20-30%, current estimates of PID prevalence are considered reliable for two reasons - the relatively low number of cases and

the possibility of misdiagnosis due to the similar symptoms of other diseases such as repeated infections.

Risk of PID

Patients with PID are usually healthy at birth because they are protected by maternal immune system. Nevertheless, they are highly susceptible to various infections after birth. If untreated, PID may result in fatal condition within a couple of years after birth due to repeated infections or even due to inoculation by a recommended live vaccine. The age at diagnosis is significantly associated with survival of patients. In the instance of common variable immunodeficiency (CVID), a major type of PID, a 1-year delay of diagnosis increased the risk of death by 1.7% every year [7]. The delay of diagnosis was also associated with the likelihood of developing lymphomas or solid tumors, supporting the importance of the early diagnosis and intervention [7].

Diagnosis and treatment of PID

PID is usually suspected when the patient suffers from repeated infections, unless the diagnosis is made immediately after birth. For example, recurrent sinopulmonary infection, sepsis and CNS infection are associated with antibody or complement deficiency. Recurrent infections on skin or internal organs are associated with phagocytic cell defects. If PID is suspected, serum immunoglobulin and complete

blood counts help evaluate and differentiate potential immunodeficiency and its cause. If a phagocytic cell defect is suspected, reactive oxygen produced from phagocytes is measured in a respiratory burst assay. If a T cell defect is suspected then cytokine production, T cell proliferation, TCR Rearrangement Excision Circle (TREC) and TCRV β chain usage are analyzed [8].

Although PID is life-threatening, allogeneic bone marrow transplantation (BMT) or hematopoietic stem cell transplantation (HSCT) can provide long-lasting protection. If a well-matched family donor is available, stem cell transplantation is usually successful [9]. On the other hand, introduction of normal cDNA into the PID patients stem cells with mutation can rescue the development of normal hematopoiesis. Thus, gene therapy offers itself as another potential option in addition to stem cell transplantation [10].

In 2008, newborn screening for severe combined immunodeficiency (SCID) using the TREC assay was introduced in the United States and later, in 2010, SCID was included in the “Recommended Uniform Screening Panel” for newborn babies. Survival was over 90% for SCID infants after stem cell transplantation, immunoglobulin treatment or gene therapy, once again supporting the importance of early diagnosis and treatment [11].

Box2

DiGeorge syndrome DiGeorge syndrome patients have a hemizygous deletion in chromosome 22q11.2 region (90% of patients) or in chromosome 10p13 region. This leads to defective development of the third and fourth pharyngeal pouches, resulting in thymic and parathyroid aplasia or hypoplasia together with congenital heart disease and aortic arch abnormality such as truncus arteriosus. Although T cell proliferation is normal after stimulation in most of the DiGeorge syndrome patients, small fragmented thymi may be found in multiple ectopic locations [12, 13].

Omenn syndrome Omenn syndrome (OS) is due to several genetic defects for which T and B cell development is partially impaired, leading to small numbers of T and B cells in peripheral blood. These genetic defects include mutations of Rag1/Rag2, Artemis, IL7R α , DNA ligase IV and IL2R γ . OS patients have a limited TCR repertoire and oligoclonal expansion of autoreactive T cell clones. These autoreactive T cells induce autoimmune disorders in various organs. OS patients show thymic aplasia or hypoplasia due to limited T cell development [14, 15].

Within the Primary Immunodeficiency Disease (PID) classification, common variable immunodeficiency (CVID) is the most common type in adults. CVID is characterized by hypogammaglobulinemias with heterogeneous causes. Generally, CVID patients experience recurrent infections in the respiratory and gastrointestinal tracts and autoimmune abnormalities occur in about 30% of CVID patients [16]. Immunoglobulin replacement therapy and antibiotics treatment are significantly effective for infections but not for the non-infectious autoimmune disorders such as rheumatoid arthritis, lupus and inflammatory bowel disease. This paradoxical manifestation of autoimmune disorders is also observed in various types of PID such as DiGeorge syndrome and Omenn syndrome. The abnormal T cell development in thymus has been suspected as the main culprit of this peculiar coexistence of autoimmune disorders and immunodeficiency in these PIDs [16].

The association of impaired T cell development, reduced thymus size and autoimmune disorders in CVID raises an interesting question how T cell development affects thymic development and subsequent removal of autoreactive T cells in thymus. With the development of new mouse models, interdependence between T cells and thymic epithelial cells (TECs) in the thymic development, and its importance in central tolerance has been proposed.

Lack of mTEC and AIRE⁺ cells in PID patients

Thymus development is a tightly interconnected developmental process of two parallel differentiation processes; T cell differentiation of bone marrow-derived precursor cells and thymic stromal cell differentiation derived from the third pharyngeal pouch dissociated from the third cleft ectoderm [17]. Precursor T cells migrate through the complex thymic epithelial network along with chemokine cues for positive and negative selections (Figure 1) [18].

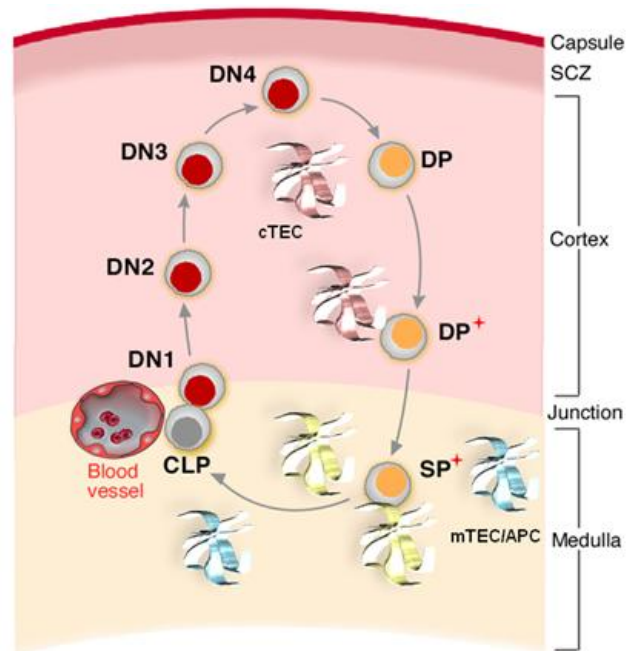


Figure 1. Thymic precursor cells enter the thymus through the blood vessels around corticomedullary junction. They move toward thymic capsule where they rearrange TCR genes. Once they have functional TCR on their surface, they scan peptide-MHC complex on cTEC in the cortex. If they are stimulated by self peptide-MHC complex, they express CD69 and move toward medulla. Aire⁺ mTEC express peripheral tissue-specific antigen and present on their surface or provide them to other antigen presenting cells for cross-presentation for the negative selection of putative autoreactive T cells. DN: double negative; DP: CD4⁺CD8⁺ double positive; SP: CD4⁺ or CD8⁺ single positive; APC: antigen presenting cells; cTEC: cortical thymic epithelial cells; mTEC: medullary thymic epithelial cells; SCZ: subcapsular zone. Red cross marks on DP and SP indicates activated CD69⁺ thymocytes

At a late developmental period, fetal liver-derived hematopoietic stem cells migrate into the thymic anlage and induce thymic organogenesis. Early T cell development stages are classified based on the expression pattern of cell surface CD molecules such as CD4 and CD8. At initial differentiation stage, thymocytes are double negative (DN) because they express neither CD4 nor CD8. Based on distinct expression patterns for CD25 and CD44, the DN thymocytes are further divided into 4 sequential developmental stages, DN1 (CD44⁺CD25⁻), DN2 (CD44⁺CD25⁺), DN3 (CD44⁻CD25⁺) and DN4

(CD4⁺CD25⁻). In the outer cortex of thymus, DN thymocytes express TCR β chain and become CD4⁺CD8⁺ double positive (DP) thymocytes which fill the most of the thymic cortex. At this DP stage, thymocytes express TCR α chain and scan various self-peptide-MHC complex on thymic epithelial cells and antigen presenting cells. If TCR $\alpha\beta$ complex has appropriate affinity toward particular self-peptide-MHC complex (“positive” selection), the thymocytes express CD69, an early activation marker, and move into the thymic medulla where they further scan peripheral tissue-specific peptide-MHC complex. If TCR $\alpha\beta$ complex does not have a strong affinity toward peptide-MHC complex expressed in the medulla, the thymocytes finish the differentiation and become mature CD4⁺ or CD8⁺ single positive (SP) thymocytes.

The thymic cortical epithelial network develops concomitantly with the early DN stage thymocyte development. This has been clearly demonstrated by the disorganization of the thymic cortex in human CD3 ϵ transgenic mice where most of thymocytes are arrested at the DN1 stage but thymic cortex is normally differentiated in Rag2^{-/-} mice where thymocytes are arrested at the DN3 stage [19]. These animal data suggests that DN2 or DN3 thymocytes do induce the differentiation of thymic cortical epithelial cells but DN1 thymocytes may not have this capacity.

Thymic medullary epithelial network develops along with the SP thymocyte development. Thus, mice lacking mature T cells have neither thymic medullary epithelial cells nor AIRE expression [20, 21]. On the other hand, the transplantation of mature SP T cells induces thymic medullary epithelial cell development in SCID mice lacking thymic medullary structure [22]. After positive selection of DP thymocytes, maturing SP thymocytes migrate into the thymic medulla in response to CCL19 and CCL21 (CCR7 ligands) expressed from thymic medullary epithelial cells for the negative selection of putative autoreactive SP T cells [23]. In *CCR7*^{-/-} mice or *plt/plt* mice lacking CCL19/CCL21, maturing SP thymocytes lose the directional migration and induce scattered small thymic medullary structures across the thymus. These

results support the hypothesis that thymic medulla development begins with proper T cell development.

Evidence in PID animal models

When the T cell development is partially impaired, a small number of autoreactive T cells may avoid negative selection by thymic medullary T cells and proliferate extensively due to the presence of cognate self peptide-MHC complex in the periphery, resulting in autoimmune disorders. The loss of thymic medullary epithelial cells results in loss of AIRE⁺ thymic medullary epithelial cells which present peripheral tissue specific antigen to maturing SP thymocytes for deletion of autoreactive T cells [21].

In OS model animals such as *Rag1*^{S723C/S723C}, *Rag2*^{R229Q/R229Q} and *ligIV*^{ts/ts} mutant mice, immune dysregulation was also associated with impaired maturation of thymic epithelial cells due to partial impairment of T cell development [24, 25]. The leaky maturation of small number of T cells expanded in these animals resulting in oligoclonality in TCR usage. The OS animal models showed reduced circulating B cells and peripheral eosinophilia in addition to the oligoclonality of T cells. Molecular analysis with several markers clearly proved a severe reduction of cytokeratin5⁺ thymic medullary epithelial cells in OS animal models. [25]. This abnormal thymic medulla was associated with reduced thymic expression of Aire and markedly reduced numbers of natural Treg cells and NKT lymphocytes [26].

Treg cells are essential to control immune response to prevent autoimmune disorder and other inflammatory diseases. The impaired development of Treg cells in humans leads to the serious autoimmune disorders such as IPEX syndrome [27, 28]. Treg cells can be divided in two major subsets: natural Treg cells (nTreg; (CD4⁺CD25⁺CD127^{dim/low}Foxp3⁺)) and induced Treg cells (iTreg). Although it is not clear yet the relative contributions of nTreg and iTreg to immunoregulation, recent animal experiment results proved that nTreg and iTreg have distinct roles and act in concert for efficient

immunomodulation [29, 30]. In a *Foxp3*-deficient mouse model, the reconstitution of either nTreg or iTreg partially rescued mice from the disease whereas the transplantation of both nTreg and iTreg prevented more efficiently than that of each subset in isolation [31]. This complementation of nTreg and iTreg functionality may reflect the different ontogeny of these cells leading to development of differing TCR repertoires [31]. nTreg develop in the thymus through high avidity interaction between TCR and self-peptide MHC complex whereas induced Treg cells develop in the periphery [32]. Thus, the reduced nTreg number may be another cause of severe autoimmune disorders in OS animal models.

Evidence in OS patients

Recently, the analysis result of TCR β usage in hypomorphic SCID patients with both immunodeficiency and autoimmune disorders supported that autoimmunity in these patients is associated with the restricted TCR repertoire [33].

OS patients showed unclear corticomedullary demarcation and lacked Hassall' corpuscles, terminally-differentiated epithelial cells, in the thymus. The expression of AIRE and AIRE-dependent tissue-restricted antigens is severely reduced [34]. In addition, nTreg cells from OS patients showed impaired suppressive activity on

comparison with similarly phenotyped cells from healthy donors [35], a result consistent with that observed in the OS animal models.

The pediatric DiGeorge syndrome patients with 22q11.2 deletion showed similar dysregulation of T cell compartment such as reduced thymic T cell development, lower T cell number in periphery, oligoclonal expansion of CD4 T cell populations and markedly reduced Treg cell numbers [36]

Anti CD3 ϵ mAb injection as an therapeutic option for PID patients with thymic hypoplasia

PID has been treated with stem cell transplantation to rebuild the whole immune system [9]. If it is difficult to find HLA-matched stem cell or bone marrow donor, gene therapy is another option for PID treatment [10]. In the instance of hypomorphic mutation of *Rag* genes, gene therapy in a preclinical study provided poor reconstitution of immune system due to inadequate *Rag* gene expression [37]. Instead, the injection of anti-CD3 ϵ antibody may be considered as a therapeutic option since anti-CD3 ϵ antibody can induce thymic organogenesis along with the induction of T cell development from DN thymocytes by crosslinking TCR complex on immature thymocytes [38].

Box 3. Aire

Aire is a transcription factor which induces ectopic promiscuous expression of a large number of genes normally expressed in peripheral tissues. Aire⁺ mTEC induces negative selection of T cells that are specific to peripheral tissue specific antigens as well as the differentiation of nTreg cells that represses T cell activation in periphery. The deletion of Aire results in defective negative selection of autoreactive thymocytes, which escape into the peripheral tissue and induces significant autoimmune responses. Aire is a proapoptotic gene which is expressed at the final maturation stage of mTEC. The death of Aire⁺ mTEC may promote cross-presentation of the peripheral tissue-specific antigens encoded by Aire-induced transcripts in mTEC [40, 41].

In TCR β ^{+/+} *Rag2*^{-/-} mice, T cell development is blocked at DP stage due to the lack of TCR α chain and thymus has cortex without medulla due to the lack of maturing SP T cells. The injection of anti-CD3 mAb activated DP thymocytes and induced ER-TR5⁺ UEA1⁺ cytokeratin5⁺ cytokeratin8⁻ thymic medullary structures [39]. In a *Rag2*^{R229Q/R229Q} OS mouse

model, the injection of anti-CD3 ϵ antibody activated DP thymocytes and induced UEA1⁺ cytokeratin5⁺ cytokeratin8⁻ thymic medullary structure [25]. Thus, the injection of anti-CD3 ϵ antibody may be applicable to other PID diseases associated with impaired thymic development to control autoimmune manifestations via facilitating negative selection of autoreactive

thymocytes and development of natural Treg cells [38].

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