Thymus

The Site for Development of Cellular Immunity

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The immune system protects our bodies against infections and cancers. This review introduce readers to the thymus – a primary lymphoid organ – which is the site of development and maturation of functional T lymphocytes. Progenitor stem cells arise from the bone marrow and undergo sequential development in the thymus where non-self-reactive T cells are selected. Subsequently, post selection and maturation, the mature T cells exit the thymus as naïve T cells into the peripheral lymph nodes. Studies on the thymus are likely to enhance our understanding of T cell development and reduce disease burdens by improving the cellular immune response.

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1. Introduction

The immune system is one of the most complex and evolutionarily developed systems in our body. It functions to eliminate foreign components and pathogens that invade our body throughout life and thus maintains good health. Diverse cells make up the immune system and act synergistically to fight infections. There are two chief arms of the immune system – innate and adaptive (*Box* 1). Innate means inborn and refers to the components of the immune system present before the pathogen has invaded the body. This system includes physical, chemical, and cellular components. The physical components comprise the skin and mucosal linings. The chemical barriers include acids present in the stomach and antimicrobial molecules like defensins and cytokines such as interferons. The leukocytes namely neutrophils, macrophages, dendritic cells, and natural killer cells make up the cellular components of the innate immune system. These cells

Keywords

Cellular immunity, T cell development, thymic hormones, thymic atrophy.

B lymphocytes arise and mature in the bone marrow and foetal liver.

express the Pathogen Recognition Receptors (PRRs) which bind to the Pathogen-Associated Molecular Patterns (PAMPs) – the molecular motifs found in pathogens – and initiate effector pathways to lower infections [1].

Among the more significant evolutionary steps in the development of the immune system is the appearance of the adaptive immune system in jawed vertebrates. The hallmarks of the adaptive immune system that distinguish it from innate immunity are: (a) specificity (discriminating even subtle difference amongst pathogens); (b) diversity (which is generated by recombination of the B cell receptor or T cell receptor genes); and (c) memory (which on successive infections mounts a faster and enhanced immune response).

B and T lymphocytes constitute the adaptive immune system. B lymphocytes arise and mature in the bone marrow and foetal liver. The presence of a membrane-bound immunoglobulin called antibody, which acts as an antigen receptor, is the characteristic feature of this type of cell. When an antibody attaches to its complementary structure called an antigen on a pathogen, the B cell undergoes clonal expansion, wherein proliferation of the particular B cells leads to generation and differentiation of plasma and memory B cells. The plasma B cells do not express any antibodies on their surface but secrete thousands of antibodies per second per cell, to counteract the antigen. These cells do not undergo further division, and a majority of them die within a few weeks. On the other hand, most memory B cells are long-lived and express antibodies on their surface [2].

Mature T lymphocytes arise from the thymus after undergoing stringent selection and maturation processes. T lymphocytes express the T Cell Receptor (TCR) on their cell surface. The majority of T cells express the $\alpha\beta$ TCR on their surfaces. An $\alpha\beta$ TCR cannot recognize an antigen directly. It does so by binding to processed antigenic peptides presented on the Major Histocompatibility Complex (MHC) molecules by Antigen Presenting Cells (APCs). MHC molecules are of two types: class I and class II (*Figure* 1). When a TCR recognizes and binds to an antigenic

Mature T lymphocytes arise from the thymus after undergoing stringent selection and maturation processes.

Box 1. Glossary of Terms

Antigen Presenting Cells (APCs): These cells process and present antigenic peptides to T cells via their MHC molecules. Dendritic cells, macrophages and B cells are known as classical APCs.

Autoimmunity: It is an aberrant immune response against components of its own body.

Cluster of differentiation (CD): These are cell surface molecules expressed on immune cells and used for studying B cells, T cells, macrophages, dendritic cells, etc. Their functions vary from cell signalling to cell adhesion and migration.

Cortex: The outer region of a body part or an organ.

Cytokines: Small soluble proteins secreted by immune cells and acting on cells in an autocrine or paracrine manner. Their functions include initiation and regulation of inflammation, cell activation, migration, development, and differentiation.

Differentiation: It is the process by which a cell type is changed into another cell type with a specialized function. For example, during T cell activation, naïve T cells differentiate into either effector T cells or memory T cells.

Fluorochromes: Chemical compounds which upon excitation re-emit light of a specific wavelength.

Immunosenescence: It is the gradual decline in the immune system occurring naturally with ageing e.g., reduced thymic output, lower T cell activation, etc.

Lipopolysaccharide (LPS): A major membrane component in almost all Gram-negative bacteria. It is a strong stimulator of innate immunity. Cells such as macrophages and dendritic cells sense LPS with the help of receptors such as LPS binding protein, CD14 and TLR4 and undergo activation to clear off the bacterial infection.

Monoclonal antibodies (mABs): These antibodies are produced by clonal, *i.e.*, identical B cells. They recognize and bind to one unique antigenic structure, called an epitope.

Medulla: The middle region of a body part or an organ.

Memory T cells: The type of T cells that have previously encountered and responded to an antigen. These cells exist in a state of readiness and rapidly expand when they re-encounter the antigenic peptide they are specific to. This strategy is the basis of vaccination.

Continued.

Box 1. ***Continued.***

Naïve T cells: These T cells are yet to encounter an antigenic peptide presented by APCs are termed as naïve. Specific T cell markers are used to identify and study them and naïve T cells in mice express high amounts of CD62L and low amounts of CD44.

Pathogen-associated molecular patterns (PAMPs): These are conserved molecules expressed by pathogens that are recognized by components of the innate immune cells e.g., LPS, flagellin, double stranded RNA, single stranded RNA, etc.

Primary lymphoid organs: They are involved in the differentiation and maturation of immune cells e.g., bone marrow and the thymus.

Pathogen recognition receptors (PRR): These proteins recognize PAMPs and initiate an immune response e.g., TLR4 recognizes LPS and TLR5 binds to flagellin.

Secondary lymphoid organs: These are the sites where T cells encounter the antigenic peptides presented by APCs and initiate an immune response e.g., tonsils, spleen, mesenteric lymph node, mucosa-associated lymph nodes, etc.

Cytotoxic T cells (T_C): The $CD8^+$ T cells upon activation differentiate to become cytotoxic T cells, which kill target cells.

T helper cells (T_H): These T cells express the CD4 T cell co-receptor. Upon activation, these cells produce cytokines and influence activation of macrophages, B cells and T_C cells.

Thymopoietic: The ability of substances or biomolecules to increase the thymic output. This is achieved by either improving the cellularity of the thymus or its architecture or both e.g., antioxidants, zinc, etc.

Transcription factors: These proteins are involved in regulating the expression of genes. They have DNA-binding domains which bind to either regulatory or promoter regions of the genes, thus modulating gene expression.

peptide loaded on an MHC, the T cell proliferates and differentiates into effector and memory T cells. Broadly, there are two types of T cells – T helper (T_H) cells and T cytotoxic (T_C) cells. These cells are differentiated on the basis of T cell co-receptors expressed on their surfaces.

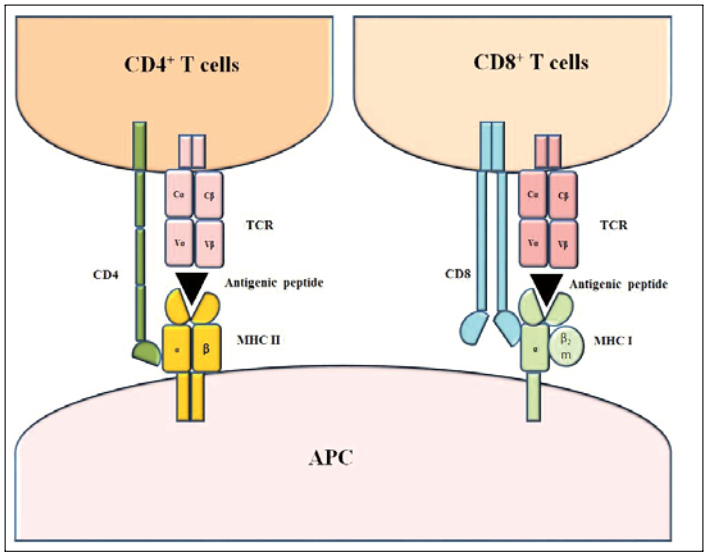


Figure 1. Structure of MHC-peptide-TCR complexes. The TCR on T cells recognizes self or antigenic peptides presented by the MHC molecules on cells. The MHC class II molecule is made of an α and a β polypeptide chain and is exclusively expressed on specialized APCs, which are the dendritic cells, macrophages, and B cells. MHC class II molecules present peptides to $CD4^+$ T cells. MHC class I molecule consists of an α polypeptide chain and a β_2 microglobulin chain. MHC class I molecules are expressed on all the nucleated cells and present peptides to $CD8^+$ T cells.

CD4 is expressed on T_H cells, while CD8 is present on T_C cells. These co-receptors facilitate optimum activation, of T cells. True to their name, post activation, the T_H cells aid in the activation of other cell groups such as B cells, T_C cells, macrophages, etc. On the other hand, the T_C cells, post activation, proliferate and differentiate into effector and memory cells. The effector cells are called Cytotoxic T Lymphocytes (CTLs) which help in clearing off cells which are either virus infected, cancerous or identified as non-self, while the memory T cells mount a hastened immune response in response to re-infection or infection post effective vaccination [3].

The generation and development of B and T lymphocytes occur in the lymphoid organs. Functionally, lymphoid organs are of two types namely primary and secondary lymphoid organs. The bone marrow and thymus are the primary lymphoid organs where the B and T lymphocytes arise and develop. Lymph nodes, spleen, and mucosa-associated lymphoid tissues are the secondary lymphoid organs where the lymphocytes encounter antigens and mount an immune response. In this article, a general overview of the thymus, its functions, and importance in immune responses are described in detail. Complications resulting due to the lack of the thymus or its dysfunction are also discussed.

2. Structure of the Thymus

The thymus is packed by a three-dimensional stromal cell network which is mostly composed of epithelial cells, dendritic cells, and macrophages.

The thymus is located above the heart (*Box* 2); it is a bilobed structure and has a thin connective tissue capsule which encloses both the lobes. Distinct thymic lobules are defined by trabeculae, which extend from the capsule. The cortex occupies the outer region of a lobule and is densely packed with immature, developing thymocytes. On the other hand, the medulla is present in the inner region of the lobule and contains the more mature

Box 2. J F A P Miller: Discoverer of the Importance of the Thymus

Jacques Francis Albert Pierre Miller was born in Nice, France in the year 1931. He grew up in Switzerland, France and China. Post World War II, his family shifted to Sydney. He pursued his PhD in the University of London where he studied pathogenesis of lymphocytic leukemia, a type of cancer in which the bone marrow produces excess lymphocytes. In 1961, Miller discovered the function of the thymus, which was still a medical mystery. While studying leukemia-inducing virus infection in mice, he observed that neonatally thymectomized (removal of thymus at birth) mice had severely atrophied lymphatic system and were unable to reject foreign skin grafts. The removal of thymus of mice at birth before the leukemia virus was injected lead to death of mice at an early stage because of their vulnerability to infections. He also observed that when thymic implantation was performed 6 months after thymectomy (post 1 month after birth) there was a restoration of symptoms of leukemia (leukemogenesis), where mice were inoculated with leukemia virus at birth. He concluded that the thymus produce the ancestor cells which migrate and establish a robust immune system [5].

In 1968, Miller's laboratory discovered that the lymphocyte population could be differentiated into two distinct populations: T cells and B cells. From his experiments he concluded that cells derived from the thymus interacted with the antigen and aided in the differentiation of the antibody producing cells [6].

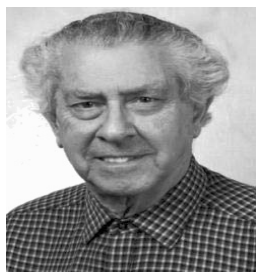


Figure A. J F A P Miller
(Credit: www.science.org.au)

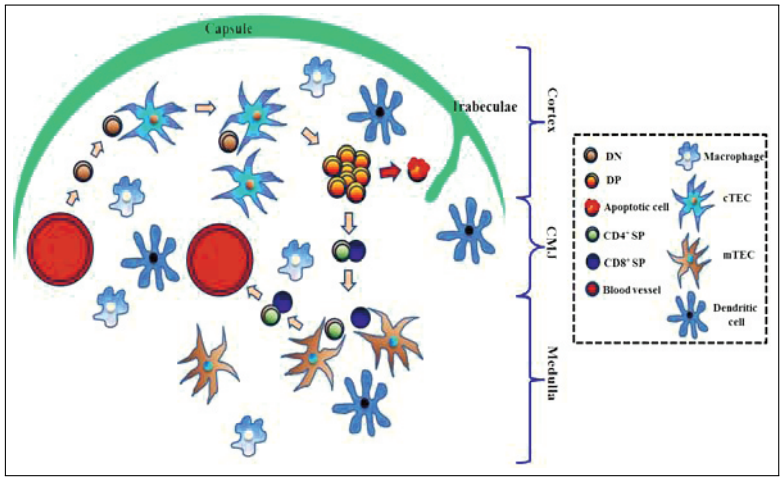


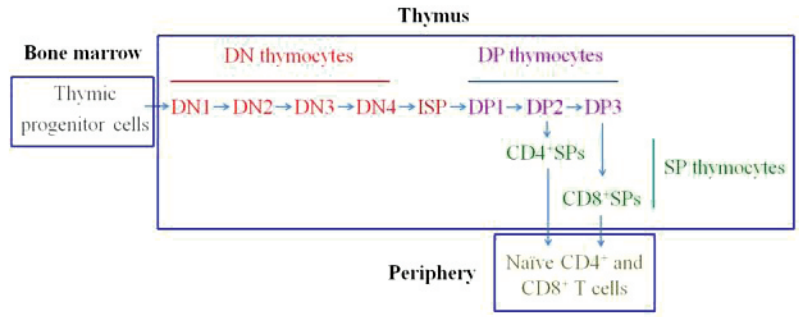
Figure 2. Structure of the thymus and development of thymocytes. The capsule covers the thymus, while structures called trabeculae divide the thymus into lobules. The thymus comprises of spatially demarcated zones: inner cortex, intermediate corticomedullary junction, and outer cortex. From the bone marrow, the thymic progenitor cells, $CD4^{-}CD8^{-}$ double negative (DN) thymocytes arrive into the thymus via vasculatures present in the CMJ. These cells give rise to $CD4^{+}CD8^{+}$ double positive (DP) cells, which in the cortex interact with mainly the cTECs. After positive selection, the DP cells produce the $CD4^{+}/CD8^{+}$ single positive (SP) cells. These cells migrate towards the medulla of the thymus and undergo further selection and maturation upon interaction with mTECs. Lastly, the mature and immunocompetent T cells exit the thymus into the periphery.

cells. The medulla and the cortex are demarcated by a vascularized corticomedullary junction. The thymus is packed by a three-dimensional stromal cell network which is mostly composed of epithelial cells, dendritic cells, and macrophages. This network is indispensable for the development of thymocytes, as the maturation of thymocytes necessitates cell-to-cell interactions between thymocytes and thymic epithelial cells (TECs), endothelial cells, mesenchymal fibroblasts, etc. Two types of TECs exist based on their localization in the thymus, cortical thymic epithelial cells (cTECs) and medullary thymic epithelial cells (mTECS), which are important during the selection of T cells [4] (*Figure* 2).

3. Cellular Components of the Thymus

3.1 *Thymocytes*

The development of thymocytes is sequential in the thymus (*Figures* 2 and 3). Interestingly, the delineation of specific developmental stages is possible on the basis of cell surface glycoproteins expressed on thymocytes (*Figure* 4). The thymic progenitor cells arrive from the bone marrow to the thymus and lack the expression of CD4 and CD8 on their surface, and are called double negative ($CD4^{-}CD8^{-}$ or DN). This population of immature cells can be further characterized into four subpopulations, on the ba-



	Mice (in days)	Humans (in days)
$CD4^+$ effector/memory T cells	14.78	164
$CD8^+$ effector/memory T cells	20.14	157

Figure 3. Development of T cells in the thymus and their lifespan. The thymic progenitor cells arrive from the bone marrow to the thymus. The most immature thymocytes, the DN cells undergo sequential development, from the DN1 stage through to the DN4 stage. These cells give rise to cells of the DP subset via the transitional ISP stage. At the DP stage, extensive selection occurs, where a majority of thymocytes are eliminated. Cells at distinct DP stages give rise to SP cells, which in the medulla of the thymus undergo negative selection and maturation, prior to exiting the thymus into the periphery. The most recent and accurate estimates of the mean lifespan of a mouse as well as human T cells in the periphery are mentioned [7].

sis of expression of the glycoproteins, CD44 and CD25, as DN1 ($CD44^+CD25^-$), DN2 ($CD44^+CD25^+$), DN3 ($CD44^-CD25^+$) cells, and DN4 ($CD44^-CD25^-$) cell subsets. At the DN4 stage, cells progress to the immature single positive (ISP) stage. The ISP cells are large, proliferate rapidly and are phenotypically characterized as $CD4^+/CD8^+CD24^{hi}CD3^{lo}$. These cells subsequently express both the T cell co-receptors CD4 and CD8, and give rise to the $CD4^+CD8^+$ or double positive (DP) cells. The DP cells can also be characterized further into distinct developmental stages on the basis of expression of the cell surface markers, CD5 and TCRβ/CD3, as DP1 ($CD5^{lo}$TCRβ/$CD3^{lo}$), DP2 ($CD5^{hi}$TCRβ/$CD3^{int}$), and DP3 ($CD5^{int}$TCRβ/$CD3^{hi}$). At the DP stage, mainly positive selection occurs, post which the DP thymocytes mature into $CD4^+/CD8^+$ single positive (SP) cells. More than 90% of DP cells fail to develop into SP thymocytes and undergo apoptosis as a result of thymic selection (*Box* 3). The SP cells migrate to the medulla of the thymus and undergo negative selection and maturation. Post selection and maturation, the naïve T cells exit the thymus as recent thymic emigrants into the periphery. The functioning of the thymus or the thymic output can be quantified by measuring the export of naïve T cells into the periphery. Although the estimates vary depending on the method used, approximately 1.0×10^9 mature $CD4^+$ T cells exit the thymus daily in healthy young 20-year-old individuals.

Box 3. Selection in the Thymus

Selection is the thymic process by which the non-responsive as well as the auto-reactive immature thymocytes are eliminated during development. Therefore only immunocompetent yet self-tolerant T cells are generated in the thymus. Two types of selection processes occur in the thymus – positive and negative selection. Positive selection primarily occurs in the cortex, while negative selection occurs in the medulla of the thymus. Both these compartments of the thymus contain different types of APCs, which present a self-peptide via their MHC molecules to the immature thymocytes, thereby creating a microenvironment that segregates thymocyte selection [8].

Death by neglect: Thymocytes that do not express TCRs or fail to express TCRs that can bind self-MHC-peptides are deleted.

Positive selection: DP thymocytes that express TCRs which recognize self-MHC complexes are selected to differentiate into SP thymocytes. This process ensures selection of thymocytes that can recognize self-MHCs. This process is mainly mediated by cTECs.

Negative selection: DP thymocytes expressing TCRs with very high affinity for self-antigens i.e., thymocytes expressing autoimmune TCRs are eliminated. mTECs facilitate the process.

3.2 *Other Cells in the Thymus*

As mentioned before, for selection of thymocytes to occur, TECs are required. These cells serve as APCs and present self-peptides to the developing thymocytes during the process of selection. B cells, macrophages, dendritic cells, epithelial cells, cTECs, and mTECs constitute the non-thymocyte cells of the thymus. These cells have been described briefly in *Box* 4.

4. Model Systems to Study the Thymus

T cell development is a complex multistep process in which the developing thymocytes interact with other cells of the thymus to undergo sequential development. In order to study T cell development, various methods have been devised such as thymus organ culture, application of thymic slices, and multicolour flow cytometry. Some of these methods are described below.

Box 4. Other Cells in the Thymus

B cells: Constitute ~ 0.3% of thymic cells and participate in negative selection.

Cortical Thymic Epithelial Cells (cTECS): These are arranged in a three-dimensional scaffold that interacts with DN and DP thymocytes. They express self-peptide-MHC complexes to developing thymocytes and thus mediate positive selection.

Dendritic Cells (DCs): These cells make up ≈ 0.5% of thymic cells and present self-peptides in the cortex and medulla.

Macrophages: These aid in phagocytosis and antigen presentation in the thymus.

Medullary Thymic Epithelial Cells (mTECs): These express tissue restricted antigens and mediate negative selection.

Natural Killer T Cells (NK T Cells): These share properties of T cells and Natural Killer Cells. They recognize CD1d, a non-classical MHC class I molecule, which binds lipids.

Regulatory T Cells: These are important in lowering autoimmunity and the ones in the thymus are known as natural T_{regs}.

Thymic Nurse Cells: The cTECs can form multicellular structures called thymic nurse cells which are involved in selection.

4.1 *Thymus Organ Culture*

As mentioned earlier, T cell development occurs in a three-dimensional stromal cell network. As the study of thymocytes in isolation may not provide a holistic picture of the ongoing processes in the thymus, 'thymic organ culture' has been developed to provide a means to study thymic processes *in situ*. In the foetal thymic organ culture method, the thymi (plural for thymus) are isolated from embryos of 15 days gestation and cultured in a nutrient-rich medium such as Dulbecco's Modified Eagle's Medium (DMEM). Post culture, the organ explants are disrupted, and the cells are mainly studied using flow cytometry. In another method called 'reaggregation thymic organ culture', the desired thymocytes, for

example, the DP thymocytes, can be cultured in the presence of specific thymic stromal cells, and the development of thymocytes can be monitored using a flow cytometer. In the 'hanging drop cultures', as few as a single cell is resuspended in a nutrient-rich medium, and the culture plate is resuspended such that the medium forms a hanging drop. The colonized lobes can be transferred to be analyzed immediately or cultured like foetal thymic organ culture [9].

4.2 *Flow Cytometry*

The cell surface as well as anti- and pro-apoptotic proteins are modulated during T cell development. The development of thymocytes can be traced by studying the T cell development and maturation markers expressed on their surfaces. This is routinely performed using the monoclonal antibodies (mAbs) specific to various markers. The mAbs are tagged to different fluorochromes and their expression, and in turn, the expression of the protein the mAb is specific to, is quantified using a flow cytometer. Using this method, it is possible to detect and quantify multiple populations of cells simultaneously. In addition, at a single cell level, multiple parameters can be studied together. For example, mAbs against CD4 and CD8 tagged to two different fluorochromes having non-overlapping emissions can be used to study and quantify the four thymocyte subpopulation *viz.*, DN, DP, $CD4^+$ SP and $CD8^+$ SP (*Figure* 4). Similarly, more fluorescently tagged mAbs against cell surface markers can be used to study the thymocyte subpopulations and Thymic Stromal Cells (TSCs) in detail.

5. Hormones and Cytokines Regulating Thymus Development

Proliferation and survival of lymphoid as well as non-lymphoid cells in the thymus are known to be regulated extensively by hormones [10]. Autocrine as well as paracrine, and endocrine pathways are at work *via* their receptors expressed on the thymic cells. Some of the important hormones regulating T cell development and survival are discussed below.

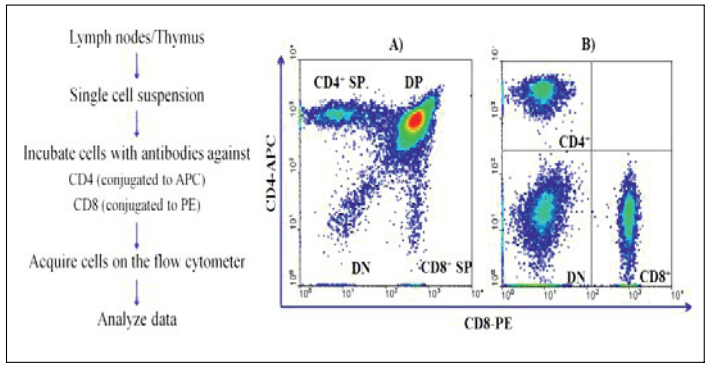


Figure 4. Flow cytometry to study immune cell populations. Single cells populations including thymocytes and lymph node cells can be studied using flow cytometry. In this example, the (A) DN, DP, $CD4^+$ SP, and $CD8^+$ SP subsets in the thymus and (B) DN, $CD4^+$ T cells, and $CD8^+$ T cells in the lymph nodes were studied using cell surface expression of the T cell co-receptors, CD4 and CD8. In order to do this, mAb against CD4 and CD8 were used to stain the surface of the thymocytes. These antibodies were tagged to fluorochromes, e.g., allophycocyanin (APC) and phycoerythrin (PE), which have emission wavelengths of 660 nm and 578 nm respectively. Therefore, post excitation by lasers, the resultant emission would be detected by distinct detectors of the flow cytometer. The results can be analyzed in the form of a density plot of CD4 versus CD8, where the cell subpopulations can be precisely quantified. Each cell is represented as a dot and is represented in one of the four quadrants of the density plots.

5.1 *Thymulin*

The thymic hormone, thymulin is secreted by TECs and is associated with intrathymic as well as extrathymic differentiation of T cells. Zinc serves as the cofactor for this peptide hormone and is therefore essential for its biological activity. Not surprisingly, supplementation of zinc in the diet leads to increase in thymi size of recently malnourished children and increases $CD4^+$ T cell counts in HIV-infected patients.

5.2 *Growth Hormone*

Growth hormone is produced and secreted by thymocytes and TECs, which also express its receptor. Growth hormone upregulates the secretion of chemokines, cytokines, and thymulin. It also enhances thymocyte migration by inducing the deposition of proteins involved in cell migration e.g., laminins and stromal cell-derived factor 1. The functions exerted by the growth hormone including thymulin induction and adhesion of thymocytes to TECS are mediated by insulin-like growth factor-1. It is also produced and its receptors expressed by thymic cells. Growth hormone also modulates the secretion of thymulin, which may contribute to its thymopoietic potential.

5.3 *Glucocorticoids*

Glucocorticoids are common mediators of thymic atropy observed during various clinical conditions. Thymocytes and TECs pro-

duce glucocorticoids and corticosterone respectively. The DP thymocytes are the main targets of glucocorticoid-induced apoptosis. Even though high amounts of glucocorticoids cause apoptosis, at low levels it rescues TCR-mediated thymocyte death, thus exerting a dose-dependent effect on thymocytes. TEC-produced glucocorticoids aid in reducing the affinity of TCR to MHC-self-peptides and thus rescues thymocytes from negative selection. Not surprisingly, deletion of the glucocorticoid receptors prior to selection leads to reduced thymus size, as the affinity of TCR to self-antigens is increased and not modulated, favouring negative selection. Inhibition of glucocorticoid action successfully reduces thymic atrophy during infections and sepsis in mice.

High amounts of glucocorticoids cause apoptosis, at low levels it rescues TCR-mediated thymocyte death, thus exerting a dose-dependent effect on thymocytes.

5.4 *Prolactin*

This protein is mainly produced by mTECs in the thymus. It acts in an autocrine manner on mTECs to induce proliferation of these cells. Thymocytes express the prolactin receptor. Defects in prolactin production or its signalling adversely affect the survival and proliferation of DN thymocytes. For example, antibodies blocking the effect of prolactin or the prolactin receptor cause a block at the DN stage of thymocyte development. Prolactin also counteracts the glucocorticoid-induced apoptosis of thymocytes.

5.5 *Neuropeptides*

Oxytocin and vasopressin are produced by the posterior pituitary lobe of the brain. These neuropeptides are also produced by TECs of the thymus. Preliminary work suggests that oxytocin receptors are critical for survival of $CD8^+$ SP cells. On the other hand, inhibition of vasopressin receptor supports the development of $CD8^+$ SP cells.

5.6 *Leptin*

Leptin is expressed in the thymus, and the lack of its receptors leads to premature thymic atrophy. Leptin and leptin receptor

Leptin is expressed in the thymus, and the lack of its receptors leads to premature thymic atrophy.

deficient mice display reduced size and cellularity of the thymus. Leptin protects thymocytes and TECs from stress-induced thymic atrophy. It has demonstrated thymopoietic potential during ageing, starvation as well as LPS-associated thymic atrophy in mice.

Patients with hyperthyroidism display increased number of thymocytes.

5.7 *Thyroid Hormones*

Patients with hyperthyroidism display increased number of thymocytes, i.e., thymic hyperplasia. In mice, treatment with the thyroid hormone triiodothyronine, (T_3) leads to increased cellularity of the thymus and enhanced proliferation, adhesion, and migration of thymocytes towards extracellular matrix molecules. These effects are mediated by nuclear receptors of T_3, expressed on thymocytes as well as on TECs.

5.8 *Sex Hormones*

Estrogen is also known to deplete the thymic progenitor cells in the bone marrow and early thymic progenitor cells in the thymus, while testosterone mediates apoptosis of DP cells via thymic GCs.

The sex hormones, androgen and estrogen, have inhibitory effects on thymocytes. Estrogen receptor signalling is required for the development of thymocytes in mice and is crucial in preventing the development of tumour growth of the thymus i.e., thymoma by inhibiting the proliferation of TECs in primary human thymoma epithelial cells. Estrogen is also known to deplete the thymic progenitor cells in the bone marrow and early thymic progenitor cells in the thymus, while testosterone mediates apoptosis of DP cells via thymic GCs. Surgical removal of testis or orchidectomy leads to increased cellularity of the thymus. On the other hand, ovariectomized mice display higher peripheral T cell numbers due to enhanced proliferation of the preexisting cells in the periphery. Sex steroid ablation has been reported to reverse age-associated thymic atrophy.

5.9 *IL7*

The cytokine IL7 displays lymphopoietic properties. It is secreted by non-haematopoietic stromal cells. DN and SP thymocytes require IL7 for survival. Mice deficient in IL7 or its receptor ex-

hibits a block in the DN stage of thymocyte development, which leads to severe thymic atrophy. Lymphopenia due to the depletion of $\alpha\beta$ T cells and the absence of follicular B cells and $\gamma\delta$ T cells is also observed.

6. Transcription Factors in the Thymus

Several transcription factors are indispensable for early thymic development and subsequent T cell selection, development, and maturation. The transcription factor FoxN1 is crucial for TEC differentiation, while Runx1, GATA3, and Ikaros are crucial for T cell differentiation and maturation. AIRE regulates the negative selection of thymocytes. These transcription factors are described in detail below.

6.1 *FoxN1*

The transcription factor, Forkhead box N1 (FoxN1) is expressed in TECs. In the foetal, as well as the postnatal thymus, FoxN1 is essential for TEC differentiation. It downregulates with age in the thymic stroma. Interestingly, forced downregulation of this factor in perinatal thymic epithelium leads to the loss off thymic homeostasis, while its overexpression in young mice slows age-associated thymic atrophy. Also, lack of FoxN1 in mice leads to the absence of hair follicles, giving the mice their name, 'nude mice' (*Box* 5).

Loss of the *FoxN1* gene leads to loss of fur and lack of proper development of the thymus. Nude mice are perfect models to be used for skin grafting as they lack mature T cells. They are useful in cancer studies as they are used to grow human tumors and can be used for anti-cancer drug testing.

Mutations in the *AIRE* gene lead to autoimmune disorders such as autoimmune polyendocrinopathy candidiasis ectodermal dystrophy, where patients possess autoantibodies against several self-antigens.

6.2 *AIRE*

The autoimmune regulator (AIRE) is critical for the maintenance of self-tolerance. Mutations in the *AIRE* gene lead to autoimmune disorders such as autoimmune polyendocrinopathy candidi-

Box 5. Difference Between Normal and Nude Mice

Normal Mice

- Normal mice have a functional thymus and a robust immune system.
- They have functional *FoxN1* which helps in proper development of hair follicles and the thymus.
- Mice reject foreign skin grafts as the adaptive immune system can discriminate foreign tissue from the self-tissue

Figure A. Normal Mice

Nude Mice

- Nude mice lack a functional thymus and lack T cells which is the cellular arm of the immune system.
- Loss of the *FoxN1* gene leads to loss of fur and lack of proper development of the thymus.
- Nude mice are perfect models to be used for skin grafting as they lack mature T cells. They are useful in cancer studies as they are used to grow human tumors and can be used for anti-cancer drug testing.

Figure B. Nude Mice

asis ectodermal dystrophy, where patients possess autoantibodies against several self-antigens. During thymocyte selection, mTECs display self-antigens or tissue-specific antigens (TSAs). These TSAs are presented to developing thymocytes during negative selection in order to eliminate self-reacting T cells. However, in AIRE-deficient mice, there is a significant reduction in the expression of TSA, which leads to the escape of self-reacting T cells from negative selection and their emergence in the periphery.

6.3 *Runx1*

Runx1 belongs to the Runx family of transcription factors, which contain the highly conserved DNA binding domain, *RUNX*. Functions of Runx1 include generation of T_{reg} cells and suppression of T_H2 differentiation. In the absence of Runx1, $CD4^+$ SPs cannot undergo maturation, and as a result, the cells are phenotypically and functionally immature. Runx1 also reduces the apoptotic sensitivity of DP thymocytes to TCR signalling, as its absence leads to the upregulation of the pro-apoptotic molecules, Fas and Bim, when TCR of the DP thymocytes are cross-linked with the anti-CD3 antibody.

6.4 *GATA3*

In addition to serving as a master transcription factor in T_H2 cells [3], the transcription factor, GATA-binding protein 3 (GATA3) is required for T cell development. Expression of GATA3 during DN stages is required for differentiation of thymocytes to the T cell lineage. It is required for optimal TCR β chain-selection, as the deletion of *Gata3* leads to accumulation at the DN3 stage of development and a resultant reduction of DN4, DP, and SP cells. At the DP to SP stage of thymocyte development, GATA3 is required for the differentiation of $CD4^+$ SP but not $CD8^+$ SP cells.

6.5 *Ikaros*

The *Ikaros* gene codes for a family of haemopoietic-specific zinc finger proteins which mediate the differentiation of erythroid, myeloid, and lymphoid cells. It also functions as a tumour suppressor. In the absence of Ikaros, there is an enormous accumulation of $CD4^+$ SP cells in the thymi of mice by the age of ~ 4 weeks. The maturation process is defective as the $CD4^+$ SP cells are phenotypically immature and proliferate rapidly and die due to low levels of the anti-apoptotic protein, Bcl-2.

7. Complications Arising in the Thymus

7.1 *Thymic Atrophy*

Thymic atrophy is the loss of cellularity of the thymus. The phenomenon was first reported by Boyd in 1932. Thymus is extremely sensitive to atrophy. It is observed to occur physiologically with age, which significantly contributes to senescence of the immune system. Factors causing age-associated thymic atrophy include accumulation of adipose tissue, loss of expression of cytokines (e.g., IL7), disruption of thymic architecture, etc. Apart from ageing, malnutrition, infection, pregnancy, cancer, cancer therapy, trauma, etc., can also cause thymic atrophy, indicating the process being stress-responsive as also demonstrated by the manifestation of thymic atrophy in astronauts returning from space flights who have elevated cortisol amounts. Not surprisingly, being stress-responsive, GCs facilitate thymic atrophy during cancer, infections, etc. Apart from GCs, sex steroids, pro-inflammatory cytokines, oxidative stress, zinc deficiency, etc., also contribute towards thymic atrophy. The influx of naïve T cells from the thymus, which contributes to enhancing the TCR repertoire in the periphery, is reduced. Consequently, the repertoire of TCR diversity in the periphery is restricted as it is maintained by the preexisting naïve and memory T cells, which results in lowered immune responses (*Figure* 5).

As the thymus occupies a critical position in the immune sys-

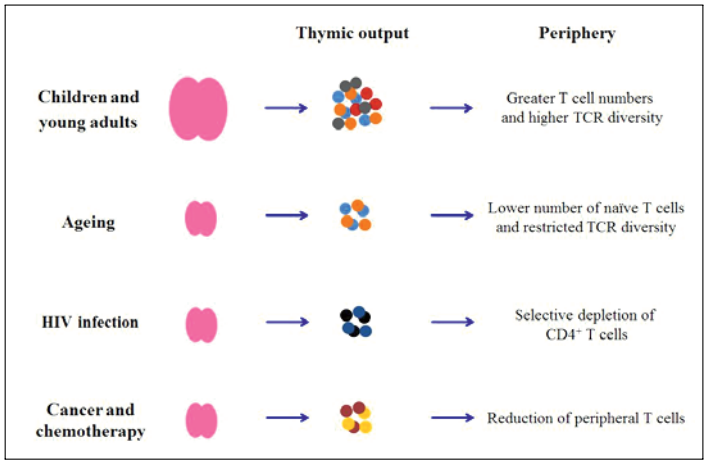


Figure 5. Thymic atrophy during ageing, infections and cancer chemotherapy affects the peripheral T cell pool. Children and young adults possess a healthy thymus with higher thymic output. This leads to greater naïve T cell numbers and increased TCR diversity in the periphery. During ageing, the thymus atrophies, lowering the output of the thymus. This consequently leads to reduced output of naïve T cells and a restricted TCR diversity in the periphery. In situations such as HIV infections, the virus directly infects and depletes thymocytes and $CD4^+$ T cells in the periphery. Cancer and chemotherapy also cause thymic atrophy and affects the thymic output adversely leading to reduction of naïve T cells in the periphery.

tem, many of the outcomes of clinical conditions depend on the functioning of the thymus. For example, post bone marrow transplantation, children display higher naïve T cell numbers, better T cell reconstitution, and lower opportunistic infections, owing to superior thymic output than adults. Similarly, HIV-infected patients on highly active antiretroviral therapy with minimal thymic tissue possess a lower number of naïve $CD4^+$ T cells and viral rebound in them is higher than those with greater thymic tissue. Conversely, younger patients on highly active antiretroviral therapy show superior $CD4^+$ T cell reconstitution. Therefore, numerous treatments possessing thymopoietic potential have been reported in attempts to reduce thymic atrophy and enhance its output, thereby reducing the morbidity and mortality linked to various clinical conditions. Zinc and antioxidant supplementation in HIV-patients and recombinant IL7 therapy and chemical sex steroid ablation in cancer patients have been successful in the reconstitution of T cell numbers.

7.2 *DiGeorge Syndrome*

Also known as chromosome 22q11.2 deletion syndrome, the congenital disorder DiGeorge syndrome affects 1:4000 births. Due to the developmental defects in the third pharyngeal pouch and the fourth pharyngeal archin, the thymus, heart, and parathyroid glands are affected. The manifestations of the syndrome are nu-

Also known as chromosome 22q11.2 deletion syndrome, the congenital disorder DiGeorge syndrome affects 1:4000 births.

merous, including immune deficiencies and cardiac anomalies. Patients with DiGeorge syndrome do not possess detectable thymus function, thus resulting in immunodeficiency. The survival rate of these patients is 0% by 2–3 years of birth. For the treatment of immunodeficiency, thymus transplantation in these patients have successfully resulted in the development of a T-cell repertoire, with functional T cells and extension of survival of the patients.

7.3 *Myasthenia Gravis*

Myasthenia gravis is a chronic autoimmune neuromuscular disease resulting in weakness of the skeletal muscles. In majority of the cases, it occurs due to antibodies against the postsynaptic acetylcholine receptor. In about 15% of the patients with Myasthenia gravis, tumour growth of the thymus i.e., thymoma is observed. In patients suffering from Myasthenia gravis, thymoma can be managed by surgery, radiotherapy, and chemotherapy. Studies on the human thymi have been facilitated by the thymi obtained from Myasthenia gravis patients post surgery.

Myasthenia gravis is a chronic autoimmune neuromuscular disease resulting in weakness of the skeletal muscles.

8. Concluding Remarks

This review is an attempt to highlight the central role of thymus in the development of the immune system and its importance during adverse conditions. Thymus is important for the generation of T cells that are responsible for cellular immunity by mechanisms involving positive and negative selection. Mice that are athymic due to the absence in expression of the transcription factor, FoxN1 also lack T cells. These mice are extensively used to study cancer and transplantation, as these mice are unable to reject the grafts. Thymus is extremely sensitive to stress, including malnutrition, infections, and cancer chemotherapy. During thymic atrophy, the thymus undergoes an acute loss in thymic architecture and cellularity. Not surprisingly, the output of thymus declines during the above conditions. Strategies to boost the output of the thymus, such as zinc and antioxidant supplementation, IL7 therapy, and

inhibition of sex steroids, have been successful in reducing the mortality and morbidity associated with clinical conditions. Further studies on the augmentation of thymic output are important as these may increase cellular immunity and consequently, reduce immunosenescence and disease manifestations.

Suggested Reading

[1] M Deobagkar-Lele, C Bhaskarla, R Dhanaraju, M Ponnusamy, D Nandi, Innate immunity and the 2011 Nobel Prize, *Resonance*, Vol.17, No.10 pp.974–995, 2012.

[2] A Ahmed, B Saha, A Patwardhan, S Shivaprasad, D Nandi, The Major Players in Adaptive Immunity – Humoral Immunity, *Resonance*, Vol.14, No.5 pp.455–471, 2009.

[3] A Ahmed, B Saha, A Patwardhan, S Shivaprasad, D Nandi, The Major Players in Adaptive Immunity 1. Cell-mediated Immunity, *Resonance*, Vol.14, No.6 pp.610–621, 2009.

[4] G Anderson, Y Takahama, Thymic Epithelial Cells: Working Class Heroes for T cell Development and Repertoire Selection, *Trends Immunol*., Vol.33, pp.256–63, 2012.

[5] J F Miller, Immunological Function of the Thymus, *Lancet*, Vol.2, pp.748–9, 1961.

[6] J F Miller, D Osoba, Current Concepts of the Immunological Function of the Thymus, *Physiol Rev*., Vol.47, pp.437–520, 1967.

[7] L Westera, J Drylewicz, I den Braber, T Mugwagwa, I van der Maas, L Kwast, T Volman, E H van de Weg-Schrijver, I Bartha, G Spierenburg, K Gaiser, M T Ackermans, B Asquith, R J de Boer, K Tesselaar, J A Borghans, Closing the Gap Between T-cell Life Span Estimates From Stable Isotope-labeling Studies in Mice and Humans, *Blood*, 122, pp.2205–12, 2013.

[8] L Klein, B Kyewski, P M Allen, K A Hogquist, Positive and Negative Selection of the T cell Repertoire: What Thymocytes See (and Don't Dee), *Nat Rev Immunol*, Vol.14, pp.377–91, 2014.

[9] G Anderson, E J Jenkinson, Fetal Thymus Organ Culture, *CSH Protoc*., pdb.prot4808.

[10] W Savino, D A Mendes-da-Cruz, A Lepletier, M Dardenne, Hormonal Control of T-cell Development in Health and Disease, *Nat Rev Endocrinol*., Vol.12, 77-89, 2016.

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