Lymphoid Development

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Introductory article

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Lymphoid cells (B cells and T cells) express antigen receptors which mediate the specific immune response. The development of lymphoid cells is tightly controlled to avoid autoreactivity and promote a diverse and useful repertoire of specificities.

Introduction

The lymphocyte compartment in mammals consists of three distinct cell lineages that include Bursa-derived lymphocytes (B cells), thymus-derived lymphocytes (T cells) and natural killer (NK) lymphocytes. In the adaptive immune response B and T lymphocytes are responsible for humoral and cell-mediated immune responses, respectively, whereas NK cells represent the major cytolytic component of innate or preformed immunity. In their role in adaptive immunity, each B and T cell acquires the expression of single unique antigen-specific receptor (B-cell antigen receptors or immunoglobulins and T-cell receptors (TCRs)) through a process of somatic deoxyribonucleic acid (DNA) rearrangement of TCR and immunoglobulin genes. This highly diverse collection of T- and B-cell receptor endows the host with a rather large spectrum of specificities (potentially more than 10^{11} discrete reactivities, also known as the 'repertoire'). The great diversity of the initial repertoires requires that autoreactive B and T cells be eliminated during their development (negative selection). In addition, αβ T cells recognize antigen as peptides bound to major histocompatibility complex (MHC) proteins. Therefore, αβ T cells must be selected (positive selection) for the ability to utilize the limited collection of self-MHC proteins as antigen-presenting molecules. These two selection steps take place in a highly specialized organ, the thymus. In the case of B cells, autoreactive B cells are eliminated and potentially useful cells are selected during their development in the specialized microenvironment of the bone marrow. There may also be some selection or licensing of NK cells for cells that express at least one inhibitory receptor for self-MHC molecules. This step would avoid harm to host by self-reactive NK cells that fail to acquire expression of such a receptor.

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B-cell Development

Overview of B-cell development

In rodents and humans, B lymphopoiesis is initially found in the embryonic yolk sac and paraaortic areas and then shifts to the fetal liver. B lymphopoiesis can also be found in the spleen and bone marrow before birth. There is a second lineage of B cells that constitutes 5% of all B cells called B1 cells, because they arise first during development in the fetal liver and omentum. In adult physiology B-1 B cells reside primarily in the peritoneal and pleural cavities and appear to be capable of self-renewal. Thus, they may not require replenishment from the adult haematopoietic stem cell (HSC) compartment. B1 B cells express a relatively restricted immunoglobulin repertoire and participate in T-independent responses that recognize self-components and common bacterial antigens. In adults, B lymphopoiesis is largely confined to the bone marrow, where it continues throughout life at a diminishing rate. **See also**: Bone Marrow

B-cell development in the bone marrow generates a large number of surface immunoglobulin-bearing immature B lymphocytes on a daily basis. These cells are generated from a relatively small number of progenitor cells that are irreversibly committed to the B-cell lineage. Committed B-cell progenitors may arise from a common lymphoid progenitor. The sequential steps of B-cell development are defined by cell size, immunoglobulin gene rearrangement

status and expression of cell surface molecules (summarized in **Figure 1**). **See also**: Haematopoiesis

The first clearly committed step in B-cell development is the early pre–pro-B stage. Early pre–pro B cells express the surface markers B220, c-kit (the receptor for stem cell factor (SCF)) and CD43 (cluster of differentiation 43). These cells comprise approximately 1% of the total number of bone marrow mononuclear cells. Early pre–pro B cells express gene products involved in the rearrangement of the immunoglobulin (Ig) H locus (e.g. recombinase-activating gene (*RAG*)-1, 2, TdT) and in the detection of a successful rearrangement at this locus (VpreB and λ5). Once the *RAG* genes are activated, early pre–pro B cells begin to rearrange their IgH loci and complete the initial step in this process (the D to J rearrangement) on both IgH alleles. These cells acquire expression of CD19 and are considered to be pro-B cells. Pro-B cells proceed to finish the rearrangement of one or both IgH alleles (the V to DJ rearrangement).

Once rearrangement is complete, the cells move into the early pre-B stage. The cells express a small proportion of their Igμ heavy chains on the cell surface in association with a surrogate or pseudolight chain complex (ψL). This multimeric complex (μ–ψL), also called the pre-B-cell receptor, consists of μ heavy chains that are covalently and noncovalently bound to immunoglobulin light chain-like proteins, VpreB and λ5. At this stage selection for productive antigen receptor rearrangement occurs, as discussed in detail below. When selection is complete, surface expression of the μ–ψL complex is lost as the cells transit from the early pre-B to the late pre-B-cell stage.

The late pre-B cell expresses μ heavy chains in the cytoplasm. During this stage of development the immunoglobulin light chain loci are rearranged. These rearrangements usually begin with the Igκ locus. If Igκ rearrangements fail to produce a functional light chain, the λ light chain locus is then rearranged. Following successful rearrangement and expression of an immunoglobulin light chain, a complete IgM is expressed at the surface in a complex with the signalling subunits Ig-α and Ig-β. This IgM surface-positive cell is termed an immature or newly formed (NF) B cell, and is not proliferating, but is subject to negative selection as discussed below. NF B cells that survive this negative selection step enter the periphery where they acquire IgD expression to become mature, follicular (Fo) B cells. Coexpression of IgD and IgM on the surface of the Fo B cells does not require further gene rearrangement, but rather is due to species-specific posttranscriptional or posttranslational regulation mechanisms.

The Fo B cell now becomes part of the recirculating pool of B lymphocytes. Fo B cells recirculate throughout the body, especially peripheral lymphoid tissues, where they can encounter their cognate antigen and respond to it by proliferating and then differentiating into effector plasma cells. Some of the cells of the responding B-cell clone avoid terminal differentiation and become memory B cells. Memory B cells are responsible for the rapid, high-affinity antibody responses made upon subsequent exposures to antigen. **See also**: Myeloid Cell Differentiation

Regulation of B-cell selection and development

There are two forces that determine the life history of a B-lineage cell: one is largely intrinsic and the other extrinsic to the B cell. The immunoglobulin gene rearrangement process operates largely in an intrinsic fashion and is the major force influencing the early life of a B cell. B cells are selected for survival only if they have successfully rearranged their antigen receptor genes. Extracellular events, such as exposure to cytokines like interleukin (IL)-7, Flt ligand or thymic stromal lymphopoietin (TSLP), appear to

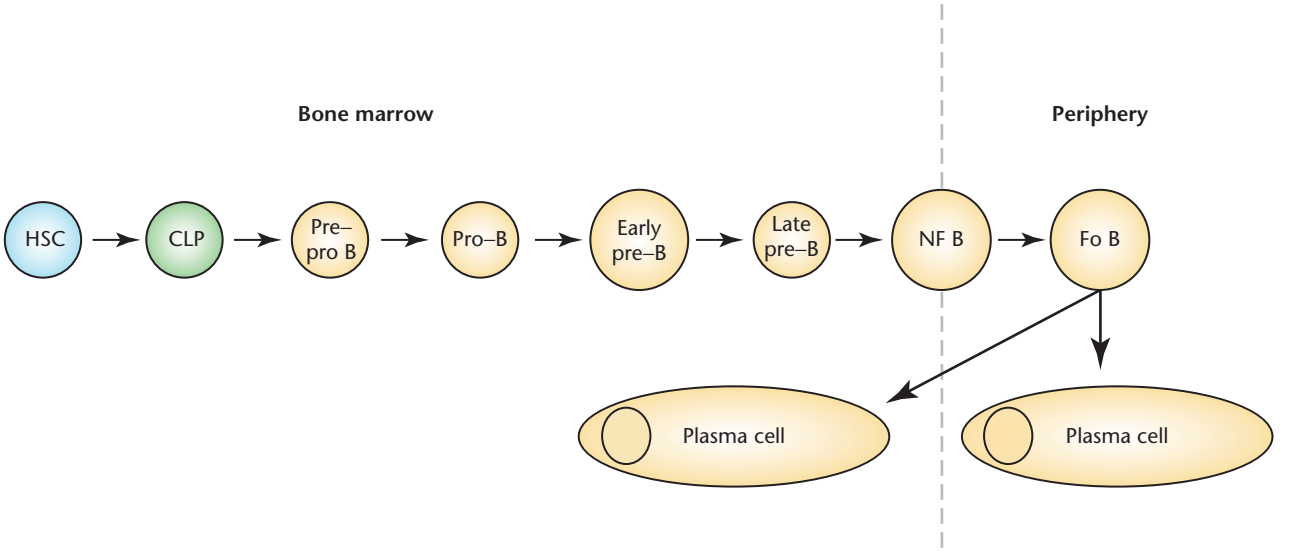

Figure 1 Schematic depiction of B-cell development. See text for details.

be required to amplify cells that have passed this selection step. **See also**: Interleukins

The first significant event in the development of B cells is their commitment from the HSC to the B-cell lineage. The molecular signals involved in this commitment step are undefined, although the transcription factors, E2A, PAX5 and early B-cell factor (EBF), seem to play a role in this process. Soon after commitment to the B-cell lineage, pre–pro B cells begin carrying out D to J rearrangements on both IgH alleles. The D to J join can take place at any of three reading frames but, interestingly, only two reading frames allow further development. It is thought that the third reading frame can lead to the expression of a truncated DJ-μ protein that interferes with development. However, the majority of pre–pro B cells will have DJ rearrangements using the other two reading frames, and become pro-B cells. These cells complete the IgH rearrangement process by carrying out a V to DJ rearrangement. If this step results in a functional μ chain, the pro-B cells assemble this heavy chain with the VpreB and λ5 chains to form the pre-B-cell receptor. This serves as a major milestone in early B-cell development that signals the successful rearrangement of the IgH locus. The pre-B-cell receptor signals by an unknown mechanism to induce cellular proliferation and differentiation at the early pre-B stage.

The next milestone for the B cell is the successful rearrangement of an immunoglobulin light chain gene. The productive immunoglobulin light chain displaces the VpreB and λ5 subunits to form a signalling complex with Igμ at the cell surface. This milestone marks the transition of the cell from the small pre-B to the immature B-cell stage. NF B cells in the bone marrow are now exposed to self-antigens. Polymeric or immobilized self-antigens have been shown to induce apoptotic death of these self-reactive cells. However, in other cases the NF B cell undergoes receptor editing upon exposure to antigen. Receptor editing is the process whereby the NF B cell replaces the self-reactive rearranged V gene with a second rearranged allele. Generally, it is the immunoglobulin light chain that is replaced during editing. NF B cells that express receptors that do not bind to self-antigens exit the bone marrow to the periphery and acquire IgG expression to become a mature B cell. **See also**: Immunoglobulin Gene Rearrangements

Selective forces continue to operate on B cells when they enter the periphery. For example, mature B cells require the expression of a functional B-cell antigen-receptor complex to enter the mature B-cell pool and to remain in this pool. The mature B cell that survives central tolerance mechanisms in the bone marrow is also subjected to peripheral tolerance mechanisms should it encounter self-antigen in the periphery. Rather than simply dying upon encountering self-antigen, these cells become anergic or unresponsive.

Perhaps the last and most important event in the life of a B cell is its encounter with a foreign antigen. If a Fo B cell has an antigen receptor that is specific for a polymeric antigen, it is likely to be independent of T-cell help (a T-cell-independent antigen). These Fo B cells then will proliferate and differentiate into plasma cells without further delay. However, these responses do not generate antibody–antigen interactions of high affinity. However, if the antigen is a complex protein, then the Fo B cell participates in the germinal centre reaction that takes place in secondary follicles of peripheral lymphoid tissues. In the germinal centre reaction, rearranged V_H genes undergo somatic mutation that alters the affinity of the antigen receptor for antigen. As a result of this process, Fo B cells that acquire high-affinity receptors are positively selected in the germinal centre, undergo further proliferation and subsequently differentiate into plasma cells or become memory B cells. Fo B cells in the germinal centre that mutate to low affinity for antigen can apparently undergo receptor editing brought on by reactivation of the *RAG* genes. Cells in the germinal centre that mutate to become self-reactive are eliminated by apoptosis. **See also**: Affinity of Antigen–Antibody Interactions; Apoptosis: Molecular Mechanisms; Immunological Memory

A distinct population of Fo B cells has been identified in recent years called the marginal zone (MZ) B cell. MZ B cells are localized to a specific region of the spleen that serves as the major antigen scavenging area of the spleen. The immunoglobulin repertoire of MZ B cells tends to be restricted and is enriched for antibodies that have specificity for bacterial antigens and senescent cell components.

T-cell Development

Organogenesis and anatomy of the thymus

In addition to developing thymocytes, the mammalian thymus consists of cells of pharyngeal endoderm and ectoderm, cephalic neural crest mesenchyme and haematopoietic origins, such as myeloid dendritic cells and macrophages. During early mouse embryogenesis, proliferative and inductive interactions between the third pharyngeal pouch endoderm and the surrounding neural crest-derived mesenchyme promote the outgrowth of the thymic primordia which is then enclosed by the surrounding pharyngeal clefts to form a rudimentary thymus. Chemoattractive mechanisms promote the migration of thymus-seeding lymphocyte progenitors into this rudimentary thymus which still lacks the histologically apparent cortex and the medulla compartments. Instead, it consists of bipotent thymic epithelial cell (TEC) progenitors that undergo a poorly defined differentiation program that allows for the generation of distinct medullary and cortical TEC subsets. It is the entry of lymphocyte progenitor cells and their 'cross talk' with the TEC progenitors that promotes and maintains TEC differentiation and their three-dimensional organization. **See also**: The Thymic Niche and Thymopoiesis

Once fully developed, the thymus is composed of five distinct microenvironments: the surrounding capsule, subcapsular cortex, deep cortex, corticomedullary junction

(CMJ) and the medulla. Developing thymocytes and thymic stromal cells, including TECs (cortical or medullary), fibroblast, dendritic cells and other haematopoietically derived stromal cells occupy each compartment. Because there are few self-renewing T-cell progenitors found in the thymus, thymocyte generation is supported by the continual influx of haematopoietic progenitors relocating from the bone marrow. This influx appears to be a controlled process that takes place periodically depending on the availability of limited thymic niches. It has been demonstrated recently that bone marrow-derived haematopoietic progenitors commit to the T-cell lineage only after arrival in the thymic microenvironment.

Overview of T-cell development

The thymic epithelium plays an essential role in T-cell development. Clear evidence of this is demonstrated by *Nude* mice, which have a mutation in the transcription factor *FoxN1* gene. Without FoxN1, TEC development is interrupted at an immature progenitor stage and the thymus never fully develops. Consequently T-cell development is absent resulting in severe immunodeficiency. Furthermore, the distinct microenvironments created by the medullary and the cortical TECs facilitate and compartmentalize the distinct phases of T-cell maturation. To complete their maturation, developing thymocytes must follow a highly coordinated migration pattern between these microenvironments. This migration is mediated by specific chemokines, such as CCL25, CXCL12 (SDF-1), CCL19 and CCL21, and integrins differentially expressed by each compartment as well as the programmed expression of the respective chemokine receptors, such as CCR9, CXCR4 and CCR7 by the developing thymocyte. The thymus-seeding progenitor enters via the CMJ, migrates through the cortex and into the outer subcapsular cortex. After reaching a specific maturation state, discussed below, the developing thymocyte progresses back towards the CMJ and into the medulla. Consistent with this, the cortex promotes early T-cell progenitor commitment and differentiation, β-selection and positive selection. In turn, the medulla supports negative selection during the late stages of T-cell development. **See also**: T-cell Receptors

In addition to being within a distinct thymic compartment during maturation, a developing thymocyte expresses distinct cell surface molecules during each phase of thymopoiesis (summarized in **Figure 2**). Specifically, more immature T-cell progenitor cells express little or no coreceptor molecules, CD4 and CD8, and are thus considered double-negative (DN) thymocytes. This non-TCR-bearing DN subset can be further subdivided into four successive maturation phases based on the expression of CD44, CD117 (c-kit) and CD25. The most primitive subset, denoted as DN1, is $CD44^+$, $CD117^+$ $CD25^-$ and is located in the CMJ or the subcapsular cortex. DN1 cells are highly proliferative and capable of maturing into either αβ thymocytes or γδ thymocytes. These cells also have the potential to generate other thymic-derived lineages such as DCs and NK cells, but have limited potential to generate B cells or myeloid cells.

Expression of CD25 marks the progression into the next maturation phase, DN2. Progenitor cells of the DN2 subset are also highly proliferative and retain limited NK and DC lineage potential. T-lineage commitment occurs during the subsequent DN3 phase, which is characterized by the downregulation of CD44 and CD117 expression. Rearrangement of the TCRβ, TCRγ and TCRδ loci mediated by RAG1 and RAG2 is detected in DN2 cells and continues predominantly in noncycling DN3 cells. Only cells that have productively rearranged their TCR loci are allowed to differentiate further, while those that have not undergo cell death. For αβ lineage progenitors, the functional TCRβ associates with the invariant pre-TCRα chain and CD3 signalling molecules to form the pre-TCR complex. The development of γδ lineage progenitors will be discussed subsequently.

The assembly of the pre-TCR complex, which self-oligomerizes and signals autonomously without ligand engagement, mediates β-selection by providing signals that promote survival, intense proliferation, cessation of TCRβ locus recombination (allelic exclusion) and differentiation into the next maturation phase, DN4. During this maturation phase, the selected cells downregulate CD25 and progress through a transient immature CD8 single-positive (ISP) stage. Subsequently, developing thymocytes upregulate both CD4 and CD8, thus marking their entrance into the double-positive (DP) maturation phase. In addition to autonomous pre-TCR complex signalling, entry into the DP maturation phase is dependent on thymus-derived factors, such as Notch signalling.

Positive and negative selection of αβ thymocytes

As DP cells, which constitute approximately 80% of the total thymus, developing thymocytes proliferate and migrate into the deep cortex where they become a quiescent DP cell and initiate TCR α-chain rearrangements. Rearrangement at the α locus is successful and thus terminated only when the α chain forms an MHC-restricted receptor when paired with the β chain, a process called positive selection. The α locus is structured such that multiple V/J recombination events can take place on the same allele, each time resulting in the excision of the prior recombined DNA thus allowing for processivity. DP cells have approximately 3–4 days, their average life span, to generate a successful rearrangement. The presence of an MHC class I or class II-restricted receptor on DP precursors provides a signal that promotes downregulation of RAG expression, long-term survival and migration into the medulla where the thymocyte can undergo negative selection.

Many studies have shown that in addition to MHC restriction, positive selection involves the combined recognition of peptide and MHC, as does T-cell activation. Both affinity and avidity between the TCR and self-peptide have been shown to be important in selection, either positive or

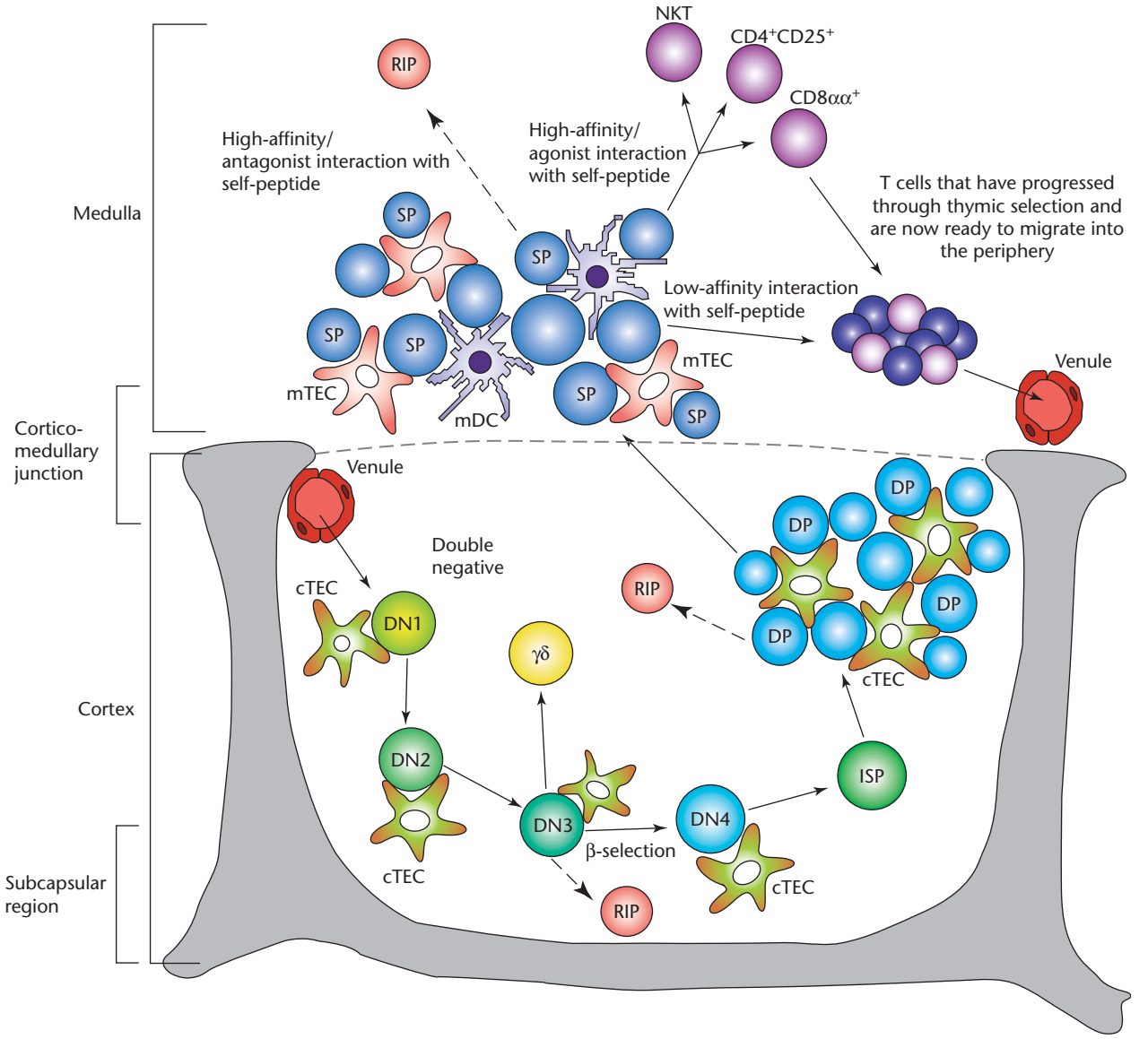

Figure 2 Schematic depiction of T-cell development. See text for details.

negative. Though the nature of the selecting peptide is not well defined, affinity measurements support the idea that TCR affinity for positive selection is lower than for negative selection. Accumulated data also supports the concept that relatively rare, low-affinity self-peptides promote positive selection, while a larger amount of any given ligand promotes negative selection. Additionally, studies have shown that the expression of coreceptors CD4 and/or CD8, which bind to MHC, contribute to TCR binding and to the resulting signal strength, thus impacting the selection choice. For example, a positively selecting ligand can become a negatively selecting ligand when coreceptors are overexpressed.

Avidity and affinity are both properties intrinsic to the selecting ligand. Extrinsic factors such as the context in which the selecting ligand is presented have also been suggested to be important in selection. Specifically, studies suggest that distinct thymic epithelial subtypes are necessary though not absolute for maximum efficiency of selection. The cortical thymic epithelium has been clearly shown to be required for positive selection in experiments using TCR and MHC doubly transgenic mice. Conversely, the requirement of a specific cell type for negative selection is unclear. *In vitro* studies have shown that susceptibility to negative selection is much higher among thymocytes in immature TCR-expressing phases. Additionally, *in vivo* TCR transgenic mice have demonstrated that DP thymocytes located in the thymic cortex undergo efficient negative selection. Incongruously, in mice with a targeted disruption of the *relB* gene, which lack medullary

epithelium and dendritic cells, potentially self-reactive thymocytes are not deleted efficiently by the remaining cortical TEC. Thus, medullary epithelium and antigen-presenting cells are thought to play primary roles in mediating negative selection.

T cells whose receptors recognize self-peptide:self-MHC complexes in the thymus too strongly undergo apoptosis, thus eliminating potentially self-reactive cells. This process, called negative selection, is mediated by several different cell types, but is driven most efficiently by bone marrow-derived dendritic cells and macrophages. In addition, other thymocytes, thymic stromal cells and TECs can cause deletion of self-reactive cells. Negative selection can occur throughout all stages of development in both the thymic cortex and medulla. Importantly, positive and negative selection does not necessarily occur sequentially. To ensure that autoreactive T cells are deleted from the mature T-cell repertoire, all self-peptides must be presented in the thymus, even peptides of 'tissue-specific' proteins. The gene product of *aire* (autoimmune regulator) has been shown to partly mediate the expression of self-peptides by stromal cells within the medulla. Lymphotoxin signaling induces the expression of AIRE. Mutations in *aire* results in an autoimmune disease known as autoimmune polyglandular syndrome type 1 or autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED).

Development of γδ T cells

In addition to the generation of αβ T cells, the thymus produces another lineage of T cells that express TCRs of the γδ type (see **Figure 2**). As mentioned earlier, the β, γ and δ locus rearrange concurrently in developing thymocytes in the DN3 phase. The decision to commit to γδ lineage is thought to be made upon the generation of a functional γ and δ chain, which would generate a functional γδ TCR, before the generation of a productive β chain. Studies suggest that the signals emanating from a functional γδ receptor are dominant over the signals generated by a pre-TCR, thus leading to γδ lineage commitment. Notch signalling is also thought to contribute greatly to this commitment. Once rearrangement results in a functional γδ TCR, all other rearrangement is halted assuring γδ lineage commitment. This allows the γδ precursor T cell to differentiate past the DN3 phase, which involves downregulation of CD25 and upregulation of CD5 and CD27. Unlike αβ T cells, γδ T cells do not express either CD4 or CD8 coreceptors and thus do not enter the DP phase. Furthermore, there is no evidence for a pre-TCR-regulated checkpoint as seen with αβ thymocytes. Instead, selection of γδ thymocytes occurs at a single TCR-dependent event. Nonclassical MHC ligands, as well as an obligate positive-selecting thymic stromal determinant have been described to mediate this selection. Though, the dependence on a common ligand that promotes γδ selection is yet unknown.

The generation of T cells along either the αβ or γδ lineage is developmentally controlled. Specifically, γδ T cells make up about 20% of double-negative cells in the adult mouse thymus but during embryonic development, γδ T cells are more prevalent, being the first T cells to appear. Moreover, the generation of γδ T cells occurs in distinct waves, with the γδ T cells in each wave populating different sites. The first wave of γδ T cells colonizes the epidermis and adopts a dendritic cell-like form. These γδ T cells are thus called dendritic epidermal T cells and are responsible for being the first line of defense against tissue damage. The second wave of γδ T cells colonizes the reproductive epithelium. Interestingly, given the large number of possible rearrangements, γδ T cells of these early waves express TCRs of essentially invariant specificity. Thus, specific V, D and J gene segments rearrange at particular times during embryogenesis. After these initial waves, γδ T cells with variable TCR specificities are produced continuously rather than in bursts together with T cells of the αβ lineage. These γδ T cells then occupy the gut epithelium as well as other lymphoid organs.

Agonist selection of regulatory T cells

Positive and negative selection in the thymus allows for the elimination of self-reactive T cells. Though this process is not fool proof allowing some self-reactive T cells to escape into the periphery and potentially mount an attack against host tissues. This is mitigated by the fact that the thymus also promotes the selection of regulatory T cells that can actively suppress self-reactive T cells. These regulatory cells, namely NKT cells, $CD8\alpha\alpha^+$ intraepithelial T cells and $CD25^+CD4^+$ T cells are specialized αβ T cells generated in the thymus that do not seem to follow the same differentiation program as αβ T cells (see **Figure 2**). Instead of being selected for by a low-affinity interaction with self-peptide, these regulatory cells require a high affinity or agonistic interaction with self-peptide for both selection and further differentiation. In addition to exhibiting regulatory activity and requiring an agonistic interaction with self-peptide, these populations display an activated or partially activated phenotype. Thus, it is not clear if such cell types are truly distinct lineages or if they are cells that are in particular activation states as a result of their response to specific MHC/self-antigens.

NKT cells are αβ T cells that also express the NK 1.1 antigen. By means of cytokine secretion, NKT cells are thought to influence the activity of conventional T cells. A distinct TCR specificity predominates among NKT cells, namely the Vα14 TCR that is selected by the nonclassical class I molecule CD1. Other TCR specificities exist among NKT cells, as made evident in conventional class I- and class II-restricted TCR transgenics. $CD8\alpha\alpha^+$ T cells are also another specialized regulatory subset of αβ T cell that make up over 50% of intraepithelial T cells. They can express either classical or nonclassical class I-restricted TCR. Owing to the fact that CD8αα homodimers can be upregulated on activated T cells, $CD8\alpha\alpha^+$ T cells are thought to represent an activated population instead of a separate T-cell lineage. In class I- or class II- restricted TCR transgenic mice, both double-negative NKT cells and

$CD8\alpha\alpha^+$ are generated and expanded when agonist ligand is presented in the thymus.

$CD4^+CD25^+$ regulatory T cells (Tregs), which emerge from the thymus as a mature and distinct T-cell population expressing FOXP3, are capable and necessary for the active prevention of autoimmune diseases that are mediated by pathogenic self-reactive T cells. In mice, Tregs are detectable in the periphery by day 3 after birth. In fact, thymectomy on day 3 results in autoimmune diseases similar to that seen in mice depleted of Tregs. These findings reflect the essential role of the thymus in the generation of these regulatory T cells. Like NKT cells and $CD8\alpha\alpha^+$ T cells, the development of $CD4^+CD25^+FOXP3^+$ regulatory T cells have been shown to require higher affinity and avidity agonist interaction between their TCR and self-peptide/MHC expressed on the thymic stromal cells, particularly cortical epithelial cells. Though, the interaction must not be so strong as to cause their deletion. Accessory molecules, such as CD28, B7 and CD40 also contribute to Treg cell generation. Mice deficient in these accessory molecules or mice treated with CTLA-4 Ig, which blocks the interaction between B7 and CD28/CTLA4, have substantially less Treg cells than wild-type mice which enhances the onset of various autoimmune diseases. Furthermore, the antigen specificity of Treg cells is as diverse as that seen among naïve T cells. It has been suggested that the broad repertoire of TCRs among naïve T cells may be duplicated in the TCR repertoire of Treg cells, with the latter having TCRs with higher reactivity to the selecting self-peptide/MHC ligand. Owing to the resulting high self-reactivity and broad repertoire, Treg cells are capable of dominantly controlling immune responses against self and nonself antigens.

Natural Killer Cell Development

Overview of NK cell development

NK cells were originally identified by their ability to spontaneously lyse tumour cells *in vivo*. They belong to the innate arm of the immune system and possess effector functions that include cytolysis of target cells and generation of cytokines and chemokines. NK cells can be distinguished from their adaptive counterparts, T and B cells, by a lack of rearranging antigen receptors. Natural killer receptors (NKR) can be broadly categorized as activating or inhibitory. Inhibitory NKR recognize classical and nonclassical MHC molecules. While activating NKR recognize MHC-like molecules that are induced upon cellular stress, viral infection or neoplastic formation. It is a fine balance, or lack thereof, of stimulatory and inhibitory signals transmitted via these receptors that dictate the function of an NK cell.

NK cells are capable of developing at multiple sites in mice and humans. NK precursors (NKP) have been isolated from the bone marrow, fetal liver, thymus, spleen and lymph nodes. These findings, however, do not prove that active development of NK cells occurs in these organs. Despite finding NKP in several organs, the general consensus is that NK cell development occurs in the bone marrow. This is because many of the cellular substrates and soluble factors required for NK development are harboured in the bone marrow. Whether NK cells leave the bone marrow fully mature is not clear. NK cells develop from HSC. The development of an NK cell from HSC can be demarcated into distinct stages based on the expression of certain cell surface markers (summarized in **Figure 3**). The first step to becoming an NK cell involves a commitment to the lymphoid lineage and loss of the ability to differentiate into myeloid or erythroid cell types. These lymphoid progenitors include early lymphoid progenitors (ELP; defined as (lineage) negative = $CD3^-CD19^-Ter119^-Gr\text{-}1^-$ and positive for c-kit and fms-like tyrosine kinase 3 (flt3)) and common lymphoid progenitors (CLP; defined as $Lin^-ckit^+IL7Ra^+$). Both ELP and CLP can give rise to NK, T and B cells. The next step to becoming an NK cell is entry into the bipotent T/NK progenitor stage (TNKP). At this stage, the progenitor cell is capable of differentiating into a T cell or an NK cell, but no longer other lymphocytic lineages. Phenotypically, TNKP can be defined as $Lin^-c\text{-}kit^+NK1.1^+$ phenotype in mice, and $CD34^+CD7^+CD1a^-$ in humans.

Acquisition of CD122 defines the next stage and this cell is now a bona fide NKP ($Lin^-CD122^+NK1.1^-DX5^-$). CD122 is the common beta chain for the IL-15 and IL-2 receptors. IL-15 has been shown to be necessary for the full maturation of an NK cell. A true immature NK (iNK) cell has been difficult to define. Broadly, iNK can be characterized by a lack of full mature NK (mNK) cell phenotypic and functional features, rather than specific markers given that this stage contains a great deal of heterogeneity among receptor expression and functional capabilities. Finally, an mNK cell possesses full effector functions and expresses a combination of inhibitory and activating receptors. These include members of the Ly49 receptors in mice, KIR family receptors in humans and CD94/NKG2 complex, NKG2D and NKp46 in both species.

HSC to NK precursor

Much remains unclear regarding the signals that regulate HSC to NKP and NKP to mNK cell. The progression of an NKP from an HSC is tightly controlled and a delicate balance of extracellular signals combined with the expression of optimal levels of essential transcription factors is critical for this process. Cell–cell contact between developing NK cells and stromal cells induces an NKP to respond to soluble factors that are produced by cells in the microenvironment. This stroma-dependent process has been corroborated by *in vitro* culture systems that are unable to produce substantial numbers of NKP from HSC in liquid culture medium, even with high doses of exogenous IL-15. Haematopoietic cells that express lymphotoxin $\alpha_1\beta_2$ ($LT\alpha$) and stromal cells that express LT beta receptor ($LT\beta R$) are known to be important for generating NK and NKT cells. Vitamin D3 upregulated protein 1 (VDUP-1) is

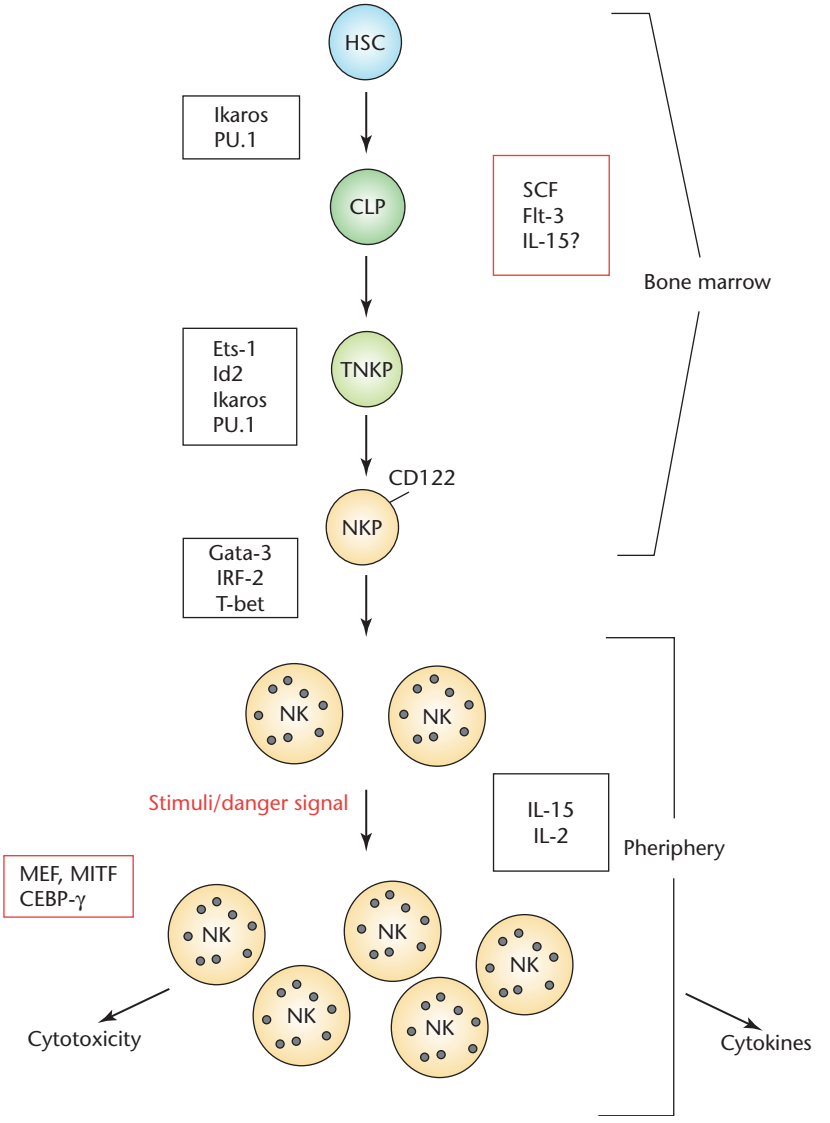

Figure 3 Schematic depiction of NK cell development. NK development occurs in the bone marrow and requires several transcription factors and soluble factors. mNK cells in the periphery are able to produce cytokines and chemokines and are cytolytic.

also an essential factor involved in NK development. VDUP-1 is a stress-response gene and mice lacking this gene have reduced NK numbers. The reduction of NK cell numbers in VDUP-$1^{-/-}$ mice is likely due to a decrease in CD122 expression.

For HSC to respond to signals that drive NK differentiation, several distinct transcription factors must be expressed. Transcription factors Ets-1, PU.1 and members of the Ikaros zinc-finger family, and the inhibitors of DNA binding, the Id proteins, all play an important role in lymphocyte development. PU.1 is critical for NKP development, but appears to be dispensable for the function and maintenance of mNK cells. Mice that are null for Ets-1 are deficient in NK cell numbers in the bone marrow, spleen and lymph nodes. Moreover, NK cells lacking Ets-1 have severely impaired cytotoxic function and cytokine generation. The Ikaros zinc-finger family transcription factor also appears to have an impact on NK cell development and may be required for expression of CD122 and Flt3 in NK cells.

NK precursor to mature NK cell

IL-15 is an essential cytokine for NK cell development and for the maintenance of NK cells in the periphery; however, the exact mechanism of IL-15 on NK cells is still an active area of investigation. Studies using IL-15 mutant mice show that NK cell maturation can occur in a somewhat normal manner, while other studies have shown that mice deficient in IL-15 have abnormal expression of Ly49 receptors. Moreover, mNK cells in IL-15-irradiated mice are

able to proliferate normally. iNK cells that are present in an IL-15 mutant mouse have normal levels of Bcl-2, an antiapoptotic protein, while mature NK cells lose expression of this molecule when transferred to an IL-15-deprived environment. Thus IL-15 appears to play different roles in iNK verses mNK cells. In fact, IL-15 may be more involved with the maintenance and proliferation of developing NK cells rather than serving as a differentiating factor.

Human NK cell maturation is also driven by soluble factors. In humans, IL-2 appears to play a role in the differentiation of the well-defined $CD56^{bright}CD16^{-}$ into $CD56^{dim}CD16^{+}$. Exactly how IL-2 affects these populations remains unclear. Since the $CD56^{bright}CD16^{-}$ population is found in the T-cell areas of the lymph node it is hypothesized that T cells may play a role in NK differentiation in humans *in vivo*. Despite this finding, IL-15, and not IL-2, is accepted to be the major cytokine involved in NK development in humans. This is corroborated by the lack of NK cells in SCID (severe combined immunodeficiency disorder) patients; these patients have an absence of the common gamma chain. Furthermore, patients lacking IL-2 or IL-2Rα have normal numbers of NK cells.

There are several transcription factors that are necessary for the differentiation of NKP into mNK cells. Studies using mice deficient in TF reveal their roles during the different stages of NK development. For example, Gata-3, IRF-2 and T-bet are implicated at the iNK cell stage, whereas myeloid ELF1-like factor (MEF), microphtalmia-associated TF (MITF) and the CAAT/enhancer-binding protein-γ (CEBP-γ) play a role in mNK cells. Gata-$3^{-/-}$ mice compared to T-$bet^{-/-}$ mice have a similar phenotype ($CD43^{lo}$ $CD11b^{lo}$) and are poor producers of IFN-γ, but both mice were normal in their cytotoxic potential. This defect in IFN-γ production may be attributed to the absence of T-bet expression, which is linked to Gata-3 expression. As mentioned, IRF-2 is also important for NK development. Mice deficient in IRF-2 have impaired ability to produce IFN-γ, and bone marrow NK cells have an immature phenotype, $DX5^{-}$ $Ly49^{+}CD43^{lo}$ $CD11b^{lo}$. These NK cells, however, appear to be cytotoxic against sensitive targets. In contrast to this, splenic NK cells from IRF-2-deficient mice have an even less mature phenotype and lack expression of Ly49 receptors entirely. For mNK cells to carry out their effector functions, MEF, MITF and CEBP-γ must be expressed. NK cells that are deficient in any of these TF develop normally, but have impaired cytotoxicity and cytokine and chemokine production.

Further Reading

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