

B and T Cell Development

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Abstract Hematopoiesis is the ordered development of the different blood cell populations from stem cells. One early branch point in hematopoietic development is the lymphoid pathway which gives rise to B and T lymphocytes and NK cells. B and T lymphocytes express antigen-specific receptors that are generated by the genetic joining of segments randomly selected from multiple loci. The cells expressing the resulting antigen receptors are subjected to a selection process for receptors with appropriate binding properties. Following the selection process, the naïve cells enter the circulation and respond when they encounter antigen able to be bound by the receptor. Once stimulated, the cells mature into functional lymphocytes in a process that is dictated by the factors and signals that are present in the environment. While NK cells can develop in the thymus, NK cells do not express antigen-specific receptors, but instead express a variety of activating and inhibitory receptors. The integration of responses from these receptors determines if the NK cell will respond. The developmental process from stem cells into mature functional cells can be interrupted at any step by genetic mutations which cause uncontrolled proliferation. Thus, an understanding of the changes important for the development of leukemias and lymphomas facilitates generation of new treatments for these diseases and also enhances our understanding of lymphocyte differentiation pathways.

The Development and Differentiation of Lymphocytes

The responses of the immune system are divided into two categories: innate immune responses and adaptive immune responses. Innate immune responses occur rapidly in response to antigen but lack specificity and immunological memory. Immunological

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memory is the ability to mount a more rapid response upon subsequent encounter with the same antigen. Conversely, adaptive immune responses are antigen-specific and generate immunological memory but take more time to develop as they require the expansion of antigen-specific lymphocytes that are originally present in extremely low frequencies. Because first encounter with antigen both increases the number and functionality of antigen-specific adaptive immune system effector cells, immunological memory is established.

Lymphocytes, a subset of white blood cells, are important components of both innate and adaptive immune responses. There are three main types of lymphocytes: natural killer (NK) cells, B lymphocytes (B cells), and T lymphocytes (T cells). NK cells are large, granular lymphocytes that serve as the main effector cells of the innate immune system. Effector functions mediated by NK cells include secretion of cytokines and other soluble factors that regulate responses of number of sub-populations of immune cells and lysis of a variety of target cells. B cells and T cells are smaller lymphocytes essential to the adaptive immune response. These lymphocyte populations use similar mechanisms of random recombination to generate a highly diverse set of antigen-specific receptors that is key to the enablement of antigen-specific immune responses. These antigen-specific receptors are called immunoglobulins (or antibodies) on B cells and T cell receptors (TCR) on T cells.

B cells initially express membrane-bound immunoglobulin. Once activated, B cells secrete immunoglobulins which are able to trigger a variety of responses once they bind to antigen. T cells are also capable of providing help to other immune cells through the expression of activation antigens that serve as ligands for receptors on other lymphocytes and/or by the production of cytokines that regulate the immune responses of these immune cells. One subset of T lymphocytes is also able to mediate cytolytic activity when activated. This chapter will present an overview of these three populations of lymphocytes, including developmental pathways, mechanisms responsible for regulation and generation of function, and how dys-regulated lymphocyte growth can result from oncogenic transformation and subsequently lead to development of leukemias and lymphomas.

Hematopoiesis

Hematopoiesis is the term used to describe the overall blood cell development process. This developmental pathway starts with hematopoietic stem cells (HSC), which are defined as cells able to both replicate themselves and generate progeny cells that can ultimately develop into more than ten different lineages. It was originally thought that receipt of specific signals directed the differentiation of HSC progeny to a particular pathway in a single step [1, 2]. Yet, recent studies suggest that the differentiation process is more progressive in nature, with cells gradually losing the ability to commit to certain lineages as they are placed in specific environments. Therefore, cells will eventually reach the point of being fully committed to a single lineage. Previous studies have shown that transferring cells from one

environment to another will allow them to develop down alternative pathways if they are not yet fully committed to a particular pathway, demonstrating the environment to which differentiating cells are exposed plays an important role in differentiation. One method of distinguishing cells at different stages of hematopoiesis involves characterization of adhesion molecule and chemokine receptor cell surface expression. This pattern of cell surface marker expression is important because it directs cells to certain environments. An alternative approach is to study the expression of cell surface receptors that respond to signals in that environment and direct the expression of regulatory factors such as transcription factors. Transcription factors, which are proteins that bind to DNA and influence gene expression, are important because they are responsible for activating loci whose products drive cells toward certain pathways or away from alternative pathways. For B and T lymphocytes, it is also important to study the stages involved in the development of the clonal antigen-specific receptors.

The initial approach to identify HSC has been to sort cells based on their cell surface markers and then test the ability of the sorted cell populations to repopulate immunodeficient murine recipients [1]. This approach identified cells expressing the cell surface marker combination $\text{Lin}^- \text{CD45}^{\text{lo}} \text{CD34}^+ \text{CD38}^+ \text{CD45RA}^- \text{CD90}^- \text{Ki67}^+$ as the cell population that contained HSC. However, this approach is limited to the analysis of the expression of cell surface markers and cannot be used to measure the ability of these cells to respond to specific stimuli. Single-cell level analysis of HSC-containing populations indicated that responses to different cytokine stimuli were exceedingly heterogeneous [3]. It was also observed that adding combinations of different cytokines often generated greater responses than a single cytokine alone. This observation is consistent with concept of heterogeneous populations within these HSC populations.

Stochastic differences may also contribute to HSC heterogeneity. Sorting HSC-containing populations based on cell cycle stage suggested that HSC in different stages produced different types of cells [4, 5]. A related experiment used single-cell mass cytometry, which permits simultaneous analysis of 34 parameters by using antibodies labeled with transition element isotopes in chelated tags [6]. Analysis of the resulting multidimensional data segregated healthy human bone marrow cell populations into a number of different populations. Using this technology, analysis of HSC-containing populations indicated that even the Lin^- population is still quite heterogeneous and that the differentiation of HSC, as well as of more differentiated cells, actually represents a continuum along which cells increasingly develop more constrained differentiative outcomes as they progress. This data suggests that the development of cells down unique hematological pathways is not the result of discrete steps, but instead a gradual transition marked by the progressive loss of differentiation options.

From HSC, the next step in the development of T and B lymphocytes is the presence of multipotential progenitor cells (MPP), which are characterized as $\text{Lin}^- \text{CD45}^{\text{lo}} \text{CD34}^+ \text{CD38}^+ \text{CD45RA}^- \text{CD90}^- \text{Ki67}^+$. MPP have lost self-renewal potential but still retain the capacity to differentiate down multiple lineage pathways [6]. In mice, MPP further differentiate into lymphoid-primed MPP (LMPP), or alternatively, to

common lymphoid precursors (CLP). Both LMPP and CLP are strongly biased toward lymphoid differentiation but have differing abilities to differentiate along other pathways (such as the myeloid pathway). The reason for the presence of these heterogeneous categories of lymphoid precursors in mice remains unclear. In humans, the cells that migrate to and populate the thymus are CLP (CD10⁺CD24⁻CD34⁺) [7]. Directing these lymphoid precursors toward the B lymphocyte or T lymphocyte pathway appears to be based on the ability to repress alternative differentiative pathways. Signaling via Notch receptors is important for repressing the development of other pathways and generating T lymphocytes. Conversely, the silencing of the Notch1 receptor signaling by the Pax5 transcription factor, along with EBF1, appears to be important in the directing lymphoid precursors toward the B lymphocyte pathway [8].

B Lymphocytes

The development of B lymphocytes in the bone marrow was recently studied using single-cell mass cytometry multiparameter analysis. The results of this study identified a progression of B lymphocyte precursors which undergo gradual changes in cell surface marker expression. These different combinations of surface markers can be used to define the different precursor populations [6]. In B cell development, Pro-B cells first give rise to Pre-B1 cells, which then subsequently mature into Pre-B2 cells. Pre-B2 cells give rise to immature B cells, which in turn lead to mature- and IL3R α ⁺-mature B cells. Some of the more dramatic phenotypic changes that occur as B cells differentiate include the loss of CD38 expression, increased expression of CD45RA and CD20, and, at the end of B cell differentiation in the bone marrow, a more modest increase of CD19 and CD123 (IL3R α). While the genetic rearrangement needed to generate heavy and light immunoglobulin chains occurs early in the differentiation of B cells, the cytoplasmic expression of μ heavy chain, the first heavy chain produced, occurs in the pre-B cells. The μ heavy chain combines with two proteins called λ 5 and VpreB to generate the pre-B cell receptor (BCR). A positive signal from the pre-BCR is needed to complete differentiation into an immature B cell expressing IgM (μ heavy chain plus light chain) on the cell surface [9].

Once the B cells leave the bone marrow, they express additional cell surface markers including CD21, CD22, and surface IgD. B cells continuously recirculate through secondary lymphoid organs and move to B cell follicles, which are concentrated areas of B cells [9, 10]. When B cells encounter antigen in the follicle, they move to the boundary between the B cell follicles and the T cell areas in order to interact with T cells. The costimulatory signal provided by CD40 ligand (CD154) expressed on activated CD4⁺ T cells binding to CD40 on B cells drives the initial proliferation of the antigen-bound B cell. Within days, the cytokines produced by activated CD4⁺ T cells dictate a switch from the μ heavy chain to the γ , ϵ , or α heavy chain constant region genes. This switch is mediated by the enzyme,

activation-induced, cytidine deaminase (AID), which demethylates deoxycytidine residues in the targeted switch recombination sequences. The resulting deoxyuracil bases are then excised by a uracil DNA glycosylase (UNG). The excision of bases triggers the recombination process, which results in the downstream heavy chain assuming the position of the μ heavy chain gene. This is the process by which a B cell initially producing IgM converts to an IgG-, IgE-, or IgA-producing cell.

Activated B cells choose one of two paths after the initial antigen and costimulation-driven activation of the B cells [9, 10]. Some of the activated B cells migrate to extrafollicular areas in the secondary lymphoid organs where they rapidly expand and differentiate into antibody-secreting plasmablasts and plasma cells. These cells provide the earliest source of antigen-specific antibodies, which are an important component of the specificity mediated by the adaptive immune system. The remaining antigen-stimulated B cells stay in the follicles where they seed germinal centers. Germinal centers are locally defined environments within the secondary lymphoid organs where B cells are able to mature and undergo affinity maturation, a process that generates higher affinity antibodies [10]. The factors that dictate which path an activated B cell will take include the B cell's initial affinity for antigen and the level of T cell help that is generated.

Histologically, germinal centers have been shown to be composed of dark zone and light zone compartments [10]. The dark zone is located next to the T cell areas and contains a high density of large, proliferating B cells. These B cells have decreased surface immunoglobulin and are called centroblasts. The B cells in the light zone, known as centrocytes, are small, nonmitotic B cells expressing surface immunoglobulin. The light zone also has a network of follicular dendritic cells, which, while not derived from hematopoietic stem cells, express a number of Fc and complement receptors that allow them to trap antigens. The follicular dendritic cells can then present these antigens to B cells. In addition to the follicular dendritic cells, there is a minor population of follicular CD4+ T cells that provide signals for germinal center B cell survival.

The movement of the B cells to all of these different sites is controlled by the expression of specific chemokine markers and production of their ligands [10]. Chemokines are chemoattractant cytokines that are able to guide lymphocytes expressing appropriate receptors toward the cell sources producing the chemokines. CXCR5 is found on naïve and activated B cells and drives cells toward B cell follicles where cells producing the CXCR5 ligand, CXCL13, are located. Once B cells are activated, they upregulate expression of CCR7, whose ligands, CCL19 and CCL20, are produced by cells in the T cell area. Cells in the germinal center also express CXCR4, which recognizes the CXCL12 ligand.

Affinity maturation of B cells takes place within germinal centers [10]. When B cells proliferate in germinal centers, they exhibit high rates of mutation in the immunoglobulin variable region. This process is termed somatic hypermutation and occurs using the same enzymes that are used for switching heavy chains. AID demethylates deoxycytidine bases and the resulting deoxyuracil bases are removed by UNG. The polymerase that fills in the missing bases is error prone so wrong bases are often added randomly. The consequence of somatic hypermutation is that a number of

immunoglobulin variants are generated. B cells expressing immunoglobulin variants that exhibit increased binding affinity to the immunizing antigen are induced to proliferate further. These expanded B cell clones then differentiate into either memory B cells or long-lived plasma cells that exit germinal centers and move to other sites in the body, such as the bone marrow. In contrast, the B cells in which the affinity maturation process induced immunoglobulin variants with decreased affinity toward the immunizing antigen undergo apoptosis. This selective process is the driving force for the increasing affinity of the antigen-specific antibodies that is observed.

Another consequence of B cell somatic hypermutation is the induction of DNA strand breaks. These mistakes appear to be the primary driving force for the development of a number of different types of B cell lymphomas. Many of the categories of B cell malignancies that have been identified are listed below [9, 11].

Malignancy	Source/origin	Markers
Acute lymphocytic leukemia	Bone marrow	CD10, CD19, CD24, TdT
Chronic myelocytic leukemia- lymphoid blast crisis	Bone marrow	CD10, CD19, CD24, TdT
Chronic lymphocytic leukemia	Blood	CD19, CD20, CD21, CD24, BCR, μ , CD5
Small lymphocytic lymphoma	Primary follicle	CD19, CD20, CD21, CD22, CD24, BCR, $\mu\delta$
Mantle cell lymphoma	Primary follicle	CD19, CD20, CD21, CD22, CD24, BCR, $\mu\delta$
Follicular lymphoma	Germinal center	CD10, CD19, CD20, CD24, BCR, $\mu\delta$
Diffuse large B cell lymphoma	Germinal center	CD10, CD19, CD20, CD24, BCR, $\mu\delta$
Burkitt lymphoma	Germinal center	CD10, CD19, CD20, CD24, BCR, $\mu\delta$
Hodgkin lymphoma	Germinal center	CD10, CD19, CD20, CD24, BCR, $\mu\delta$
Multiple myeloma	Post-germinal center	CD19, CD138
Waldenstrom macroglobulinemia	Post-germinal center	CD19, CD138
Marginal zone lymphoma	Post-germinal center	CD19, CD138
Hairy cell leukemia	Post-germinal center	CD19, CD138
Plasmablastic lymphoma	Post-germinal center	CD19, CD138

T Lymphocytes

While development of B lymphocytes occurs in the bone marrow, T lymphocyte development occurs primarily in the thymus. This means that T lymphocyte precursor cells must be able to migrate from the bone marrow through the blood to the thymus. Experimental data indicates that the interaction between the chemokine

receptors, CCR9 and CCR7, expressed on precursor cells and the thymic-produced chemokines, CCL25 and CCL19-CCL21, is essential to the process of directing these cells toward the thymus. However, the expression of these receptors does not appear to play a role in the differentiation of the thymocytes [8].

Precursor cells that settle in the thymus develop along the T lymphocyte pathway because of signals generated by the Notch1 transcription factor and the cytokine, IL-7. The role of these different signals in thymopoiesis appears to vary somewhat between mice and humans. These differences include different responses to varying levels of these respective factors, varying degrees of dependence on these two factors, and the stages at which T cell precursors respond to the factors. One of the primary roles of the signals induced by these factors is to repress the ability of early T cell precursors to differentiate along alternative pathways. However, when these early thymocytes are removed from the repressive environment of the thymus, they have been shown to still retain the ability to develop into B lymphocytes, myeloid cells, dendritic cells, and NK cells in the presence of the appropriate factors. However, as demonstrated in mice, commitment to the erythroid and megakaryocyte lineages cannot be achieved once T cell precursors arrive in the thymus, as these pathways are permanently repressed prior to the thymic arrival. Once reaching the thymus, studies suggest that the ability of T cell precursors to differentiate into B lymphocytes is rapidly inhibited, followed by somewhat later inhibition of the myeloid and dendritic cell pathways, and the NK cell pathway is the latest pathway to be repressed. Analysis of human T lymphocyte precursors indicates that these cells have already lost myeloid differentiation capacity at the time of thymic arrival.

The differentiation stages of murine thymocytes are described as follows: DN1/ETP, DN2a, DN2b, DN3a, DN3b, and lastly $\alpha\beta$ T cells [8]. These stages are defined according to the expression levels of certain cell markers, including c-kit, CD44, CD25, and the β -chain of the T cell receptor. The β -chain of the T cell receptor is first expressed in stages DN3a and DN3b and is therefore an important marker of these stages. In humans, thymocyte differentiation starts with CD10⁺CD24⁻CD34⁺ cells that mature in a stepwise fashion by first acquiring CD7 and secondly acquiring CD1a [7]. Human TCR β -chain rearrangements are initially detected in CD4⁻CD8⁻CD34⁺CD1 α ⁺ cells. Cells then begin to express CD4 but not CD8 and then subsequently express both CD8 α and CD8 β in addition to CD4. A number of studies have suggested that TCR β -chain rearrangements can occur at any of these stages. The TCR β -chain then associates with a surrogate α -chain which forms a pre-TCR. Triggering this pre-TCR results in the expansion of the CD4⁺CD8⁺ double positive cells and initiation of α -chain TCR rearrangements [12]. The process of α -chain TCR rearrangement continues sequentially on both chromosomes until (1) an α -chain is formed that is able to properly associate with the previously formed β -chain and (2) the TCR complex is able to bind to a self-major histocompatibility complex (MHC) molecule/peptide complex and transduce a signal. Receiving this positive signal allows a T cell to continue differentiating, while no signal results in death of the cell. Too strong an interaction between the TCR and self-MHC molecule/peptide complex also causes a cell to undergo activation-induced apoptosis (negative selection) eliminating the cell. Presumably, this negative selection

process removes autoreactive T cells that could contribute to autoimmunity if they were allowed to complete differentiation and be released to the periphery.

Once the naïve CD4⁺ and CD8⁺ T lymphocytes leave the thymus, they circulate throughout the body in the blood or reside temporarily in lymphoid organs (such the spleen or lymph nodes). Expression of the selectin ligand, CD62L, and lack of expression of the adhesion molecule, VLA-4, direct the migration of naïve T cells to lymph nodes. Naïve T lymphocytes are unable to migrate to tissues because they have not yet been activated. Activation is achieved once T cells encounter antigen on antigen-presenting cells (APCs), such as dendritic cells, in the spleen or lymph nodes. T lymphocytes need to receive several different signals to become sufficiently activated and undergo proliferation and differentiation. Required APC signals to T cells include presentation of antigenic peptides by MHC molecules, presentation of costimulatory ligands such as CD80/86 on the APC cell surface, and production of inflammatory cytokines such as type I IFN and IL-12.

The effector responses of CD4⁺ T lymphocytes, known as “T-helper cells,” primarily consist of the production of cytokines. CD4⁺ T lymphocytes can produce a wide variety of cytokines, but usually do not produce them all at once. Instead, CD4⁺ T cells produce a discrete set of cytokines that is determined by the combination of cytokines already present in the environment when T cell activation occurs. CD4⁺ T cells are divided into categories based on the cytokines they produce: T-helper 1 (Th1) cells, T-helper 2 (Th2) cells, T-helper 3 (Th3) cells, and T-helper 17 (Th17 cells). The cytokines produced by these subsets determine their mechanism of action. Th1 cytokines include IFN- γ , IL-2, and TNF- α , which are important in the generation of immune responses to intracellular pathogens, such as viruses. Th2 cytokines include IL-4, IL-5, IL-10, and IL-13. These are important in mounting responses to extracellular pathogens, such as worms. The presence of IFN- γ or IL-4 at the initiation of the immune response is crucial in determining whether an immune response will be skewed toward Th1 or Th2. IFN- γ promotes the production of Th1 cytokines and inhibits the production of Th2 cytokines. In contrast, the early presence of IL-4 directs the production of Th2 cytokines and inhibits Th1 cytokines. The early presence of TGF- β directs CD4⁺ cells to produce Th3 cytokines, TGF- β and IL-10, which facilitate the production of IgA in mucosal tissues. When both TGF- β and IL-6 are present early in the immune response, CD4⁺ T cells are directed toward the Th17 phenotype resulting in the production of IL-17 and are often found in inflammatory conditions.

CD8⁺ T lymphocytes, often defined as cytolytic T lymphocytes (CTLs), play a major role in the Th1 response against intracellular pathogens but have also been shown to produce a variety of different cytokines. While CD8⁺ T cells can produce Th1 cytokines when activated, the primary method these cells use to eliminate intracellular pathogens is by lysing the pathogen-infected cells. The lysis of these infected cells is accomplished through exocytosis of cytolytic granules. Main components of these granules include serine proteases called granzymes and perforin, a protein that forms holes in the cell membrane [13]. Perforin facilitates entry of granzymes into the cell cytoplasm where they are able to induce programmed cell death (apoptosis) in the target cell.

Naïve CD8⁺ T cells do not contain cytolytic granules. Once CD8⁺ T cells receive sufficient activating signals, they begin producing granzymes and perforin and packaging these proteins into granules. When an activated and armed CD8⁺ T cell encounters a cell expressing the same antigen as that which triggered its initial activation, cytolytic granules are mobilized to the point of contact and triggered to exocytose stored contents into the extracellular environment next to the target cell. Once clearance of an infectious pathogen has been accomplished, activated CD8 T cells are reduced in number via a process called activation-induced cell death. Yet, even after this contraction, the number of memory CD8 T cells specific for the pathogen remains elevated. High levels of granzyme A have been observed in these resting memory CD8 T cell populations [14, 15]. With additional encounters with antigens, the process of repeated activation and return to rest results in even higher resting levels of cytolytic effector molecules. Initially, resting memory CD8 cells express granzyme A plus low levels of granzyme B or perforin. With more cycles of activation, these cells begin to express high levels of both granzyme A and granzyme B and low levels of perforin. Eventually, high resting levels of perforin are also established. These end-stage CD8⁺ cells also are marked by the expression of NK cell markers, particularly CD57. As CD8⁺ T cells progress through these different stages, it has been found that they express an increasing number of these NK markers, which can alter how they respond to specific antigens.

Five granzymes can be found in human cytolytic granules: A, B, H, K, and M [13]. These are produced and present in varying amounts depending on cell type. Out of these five types, granzymes A and B have been most extensively studied. Granzyme B induces target cell apoptosis through cleavage of caspases that trigger the apoptotic pathway. While granzyme A may also induce apoptosis via an alternative pathway, it also contributes to inflammation by cleaving, and thereby activating, assemblies of proteins called inflammasomes [16]. Inflammasome activation triggers the cleavage of IL-1 β and IL-18 pro forms, which in turn leads to the production of active cytokines that induce inflammatory responses.

Natural Killer Cells

Natural killer (NK) cells are important effector cells in the innate response to antigenic stimulation [17]. These cells produce cytokines and also mediate cytolytic activity. The development of NK cells primarily occurs in the bone marrow, although it can occur at other sites as well. Transcription factors that appear to be important for NK cell maturation include IRF-2, Gata3, Tbx21, and D1x. At an immature stage, NK cells require a common γ -chain and the IL-2R β -chain (both components of the IL-2 and IL-15 receptor), along with the associated signaling molecules. Knockout mice studies demonstrated that expression of IL-15 or the IL-15R α -chain is essential for the development and maintenance of NK cells, as absence of either one of these molecules prevents NK cell development. In humans, no individuals exhibiting loss of IL-15 or IL-15R have been reported, but information can still be

gained from studying individuals with mutations in other proteins involved in these pathways. Such studies have indicated that, like in mice, IL-15 is the key regulator of NK cell development in humans.

Although the phenotype of NK cell precursors has not been identified in humans, CD3⁻CD161⁺CD56⁻ cells can be induced in vitro from Lin⁻CD34⁺ cells. When these precursor cells are further stimulated with IL-2 and IL-12, CD56 expression and natural cytotoxicity are acquired. NK cells also undergo a selection process that results in them expressing a varying number of inhibitory receptors depending on recognition of MHC molecules expressed in the environment during their development [18]. These inhibitory receptors are important in maintaining self-tolerance. If NK cell receptors interact with self-MHC on another cell, the kill signal will be inhibited and the self-cell will remain intact. However, if a self-MHC complex is not present, NK cell cytotoxic potential will be released. This system allows NK cells to target foreign antigens and also pathogen-infected syngeneic cells (which often downregulate expression of self-MHC).

NK cells can also develop in sites other than bone marrow, including the lymph node, thymus, and intestine [17]. In the thymus, the differentiation pathway to NK cells is the last alternative pathway lost before final commitment to T lymphocyte lineage. This has raised the question of how NK-cell-inducing signals within the thymus overcome the dominant push to become T cells. NK cells in the thymus have been found to express intracellular CD3 ϵ proteins, a phenotype not observed in the peripheral NK cells. There is evidence that NK cells can also develop in the lymph nodes and intestines. The contribution of these non-bone marrow sites to the overall number of NK cells has not been determined. Unlike NK cells that develop in the bone marrow, many NK cells that develop in non-bone marrow sites are CD56^{high}CD16^{low}. The ratio observed is 10:1 CD56^{high}CD16^{low}:CD56^{low}CD16^{high} NK cells in the lymph node and intestine, whereas the ratio of these two populations of NK cells is 1:10 in the peripheral blood and spleen. These two populations of NK cells have different primary effector functions. The CD56^{high}CD16^{low} NK cells are primarily cytokine secretors, while the CD56^{low}CD16^{high} NK cells are primarily cytolytic effector cells.

Mature NK cells leaving the bone marrow are fully armed with cytolytic granules containing granzymes and perforin. Activation of NK cells to carry out cytolytic activity is usually the result of receiving more activating signals than inhibitory signals. The balance tips toward an activating response in situations where there is a lack of self-MHC molecules. This happens when the target cells are allogeneic or have lost expression of MHC molecules because the cell has been infected or because of oncogenic transformation. Activation can also occur when an NK cell receives strong activating signals. The strongest activating signal, which is able to override all inhibitory signals, is triggered by the binding interaction between an Fc receptor and antibody-coated cells or antigen.

A number of different leukemias and lymphomas have been identified as malignant transformation of cells at all of the different stages of T cell and NK cell differentiation [11]. The transformation of the more differentiated T cell stages is reflected by the identification of a heterogeneous group of T cell and NK lymphomas.

T cell lymphomas have been divided into the cutaneous T cell lymphomas and peripheral T cell lymphomas categories [19–21]. Peripheral T cell lymphomas are a heterogeneous group of lymphomas that are often derived from cytolytic cells, including NK cells. These lymphomas occur with a relatively low frequency (<1 case/100,000). They are further divided into subcategories based on clinical features including nodal, extranodal, and leukemic. The nodal group of lymphomas includes peripheral T cell lymphomas not otherwise specified, anaplastic large cell lymphoma, and angioimmunoblastic T cell lymphoma. The extranodal group contains a number of less common types, including hepatosplenic $\gamma\delta$ T cell lymphoma, enteropathy-associated T cell lymphoma (associated with celiac disease), intestinal T and NK cell lymphomas, nasal-type NK⁺/T⁺ lymphoma, and panniculitis-like T cell lymphoma.

The leukemia group includes the adult T cell lymphoma (ATL) associated with human T-lymphotropic virus type 1 (HTLV-1). The geographic distribution of the ATL is similar to where the HTLV-1 virus is endemic, which includes Japan and the Caribbean. T cell chronic large granular lymphocyte leukemia, aggressive NK cell leukemia, and T cell prolymphocytic leukemia also are included in the leukemic subcategory.

The cutaneous T cell lymphomas are primarily comprised of mycosis fungoides and the leukemic variant, Sezary syndrome [20]. Mycosis fungoides is a lymphoma primarily comprised of mature, skin-homing CD4⁺ clonal cells producing the Th2 cytokines IL-4, IL-5, and IL-10. They usually present as patches, plaques, tumors, or generalized erythema of the skin. These malignant cells can also be found in the lymph nodes and peripheral blood. These cells lack expression of T cell markers such as CD7 and CD26 but usually express clonal TCR. Gene expression profiling of STAT4, GATA3, Plastrin-T, CD1d, and TRAIL has been found to be 90% accurate in predicating Sezary syndrome.

An understanding of the developmental and differentiation pathways of lymphocyte subpopulations including B lymphocytes, T lymphocytes, and NK cells is important for categorizing the different types of malignant transformed lymphocytes. This understanding will facilitate study of the different types of lymphomas and the mechanisms responsible for their development and provide a basis for the development of novel and better therapeutic protocols.

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