PERIPHERAL BLOOD MONONUCLEAR CELLS

MEDIATORS OF THE IMMUNE RESPONSE

Shawn P. Fahl PhD
Rachel Lane PhD, RD

DISCOVERY
LIFE SCIENCES
Science at your Service.™

Contents

Introduction

Peripheral blood is the fluid that transports key nutrients and cells throughout the body. Three important cell types are found in blood: anucleated cells, multilobed granulocytes, and peripheral blood mononuclear cells (PBMCs). The anucleated cells, namely red blood cells/erythrocytes and platelets/thrombocytes, distribute oxygen throughout the body and assist in blood clotting, respectively. Granulocytes and PBMCs, also known as white blood cells, participate in innate and adaptive immune responses. Innate immunity is quickly activated after the host is exposed to highly conserved pathogenic factors. In contrast, adaptive immunity is triggered by unique, nonhost-specific factors and offers lifelong protection against these antigens, generating quicker and stronger responses to subsequent encounters. Granulocytes, monocytes, and natural killer cells aid innate immunity, while B cells and T cells promote adaptive immunity. These two immune responses are unique but not mutually exclusive: the innate system is a critical activator of the adaptive immune system.

TABLE 1: RELATIVE PERCENTAGE OF EACH IMMUNE CELL TYPE IN PERIPHERAL BLOOD MONONUCLEAR CELLS

The relative abundance of each immune cell type within the peripheral blood mononuclear cell population was analyzed with flow cytometry.

For years, mouse models have been studied for insight into the human immune response. These studies have yielded insightful results, but several differences exist between mouse and human immunity that limit the direct clinical translation of these findings. Primary human cell samples are helpful tools for scientists transitioning from mouse to human studies.

This book is designed to help researchers investigate immune-related questions in human cells, an essential step in translational research, and discusses the general functions of various human PBMC subsets and their specific roles in tumor immunology. PBMCs primarily include T cells, B cells, and monocytes, and additional cell types, such as stem cells and dendritic cells, are present at low levels (**Table 1**). These cells can be easily isolated, via immunomagnetic or flow cytometric techniques, from human blood for further experimental analysis. Immune-related PBMCs and recent advances in relevant immunotherapies are summarized in this book.

Flow Cytometry

Flow cytometry is a useful, common technique for assessing and characterizing immune cells. This high-throughput technique enables a single cell's protein expression, size, and basic composition to be analyzed. These parameters precisely categorize cells into specific populations. For example, in the left panel of Figure 1A, the flow cytometry plot depicts CD3- CD19+ B cells (upper left quadrant) and CD3+ CD19- T cells (lower right quadrant). CD3+ T cells can be selected and further characterized as CD4+ T cells (upper left quadrant) or CD8+ T cells (lower right quadrant), as depicted in the right panel of Figure 1A. In Figure 1B, the third lymphocyte cell type, natural killer (NK) cells, are identified as CD56+ CD3- (upper left quadrant), while NKT cells, which have characteristics of both T cells and NK cells, express CD56 and CD3 (CD56+ CD3+, upper right quadrant). When high quality, well defined, pure cell populations are used for scientific studies, the data are clearer and easier to interpret, allowing researchers to be confident in the results.

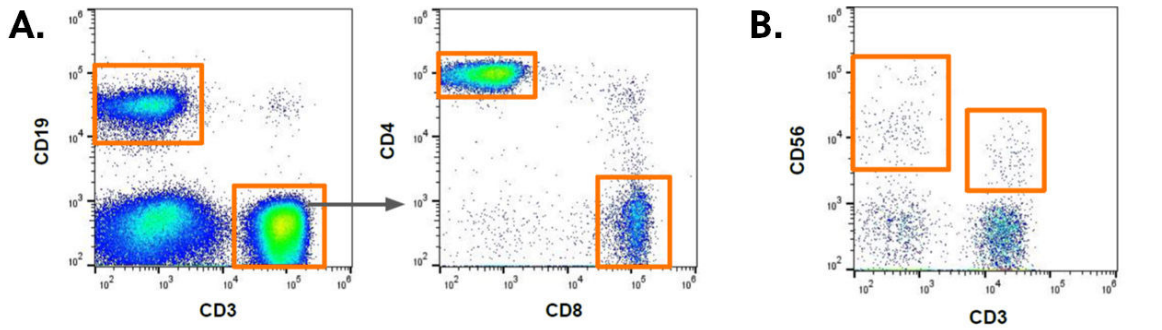

FIGURE 1: FLOW CYTOMETRIC ANALYSIS PRECISELY CHARACTERIZES SPECIFIC CELL POPULATIONS

Flow cytometric analyses of B cells, T cells, and natural killer cells are shown.

B Cells

Markers: CD19+ or CD20+ **Percent of PBMCs:** 5–20%

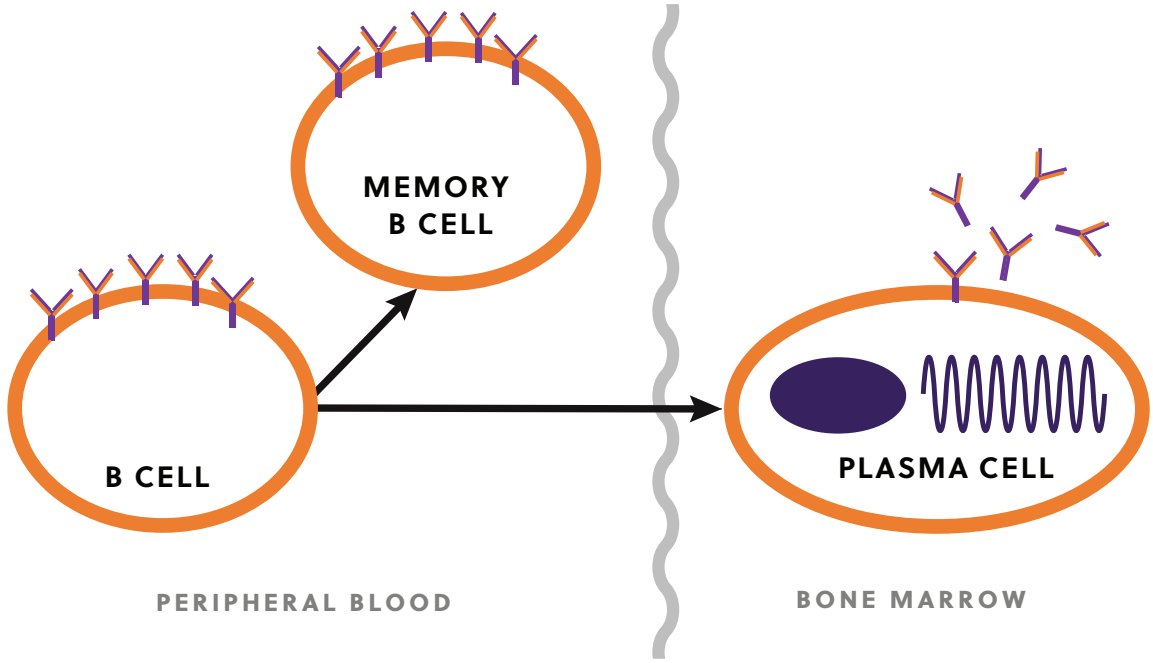

FIGURE 2: B CELLS DIFFERENTIATE INTO MEMORY B CELLS OR PLASMA CELLS

After class switch recombination and affinity maturation, B cells in the peripheral blood either differentiate into memory cells or enter the bone marrow to become antibody-secreting plasma cells.

B cells are a major component of the adaptive immune system and are the key mediators of humoral (antibody-mediated) immunity. Each B cell expresses B cell receptors (BCRs) that are composed of a variable, antigen-binding region and a constant, heavy chain region. The variable region binds one unique antigen. All BCRs on a single B cell are specific for the same antigen, but BCR specificity varies among B cells. BCR specificity is attained through V(D)J recombination, and BCR/antigen binding activates B cells. Then, activated B cells either differentiate into short-lived plasma cells that secrete surface-bound BCRs as soluble antibodies or enter a germinal center to undergo affinity maturation and class switch recombination.

In the germinal center, the BCR variable region is mutated resulting in affinity maturation, which strengthens the binding between a specific BCR and antigen. Class switch recombination, which also occurs in the germinal center, alters the immunoglobulin (Ig) isotype of the BCR constant region. All BCRs initially contain the IgM isotype, but after class switch

recombination, the IgG, IgA, or IgE isotype is expressed. Each isotype uniquely contributes to the immune response and exhibits a distinct distribution profile (**Table 2**). Class switch recombination enables the same antibody to trigger a variety of outcomes. Following class switch recombination, B cells differentiate into either plasma cells, which migrate to the bone marrow and continue to produce antibodies, or memory B cells, which are quickly activated upon subsequent exposures to the same antigen (**Figure 2**).

ROLE IN TUMOR IMMUNOLOGY

The ability of B cells to identify specific, foreign and mutated self antigens should enable this cell population to target and eradicate cancer cells. Some studies confirm this ability, indicating that B cells produce neutralizing, antitumor antibodies when stimulated by follicular helper T cells (T_{FH}) and specifically cooperate with CD8+ T cells to inhibit tumor progression.[1,2] However, other studies suggest that B cells promote tumorigenesis through cytokine production, regulatory T cell (Treg) generation and T cell suppression.[2]

TABLE 2: IMMUNOGLOBULIN ISOTYPE CHARACTERISTICS

Each immunoglobulin (Ig) isotype has a unique function and distribution pattern.

IMMUNOGLOBULIN (Ig) ISOTYPE	STRUCTURE IN BLOOD	DISTRIBUTION/FUNCTION
IgA	Monomer or dimer	Found in mucosal areas; protects against inhaled/ingested pathogens
IgD	Monomer	Mostly B cell-bound (coexpressed with IgM); secreted in upper respiratory tract; function unknown
IgE	Monomer*	Binds allergens on mast cells
IgG	Monomer	Main Ig in serum; functions in humoral immunity
IgM	Pentamer	Initial Ig on B cells; indicates recent infection

**IgE is rarely observed in monomeric form, as it is quickly bound by the surface receptors present on mast cells*

T Cells

Marker: CD3+ **Percent of PBMCs:** 45–80%

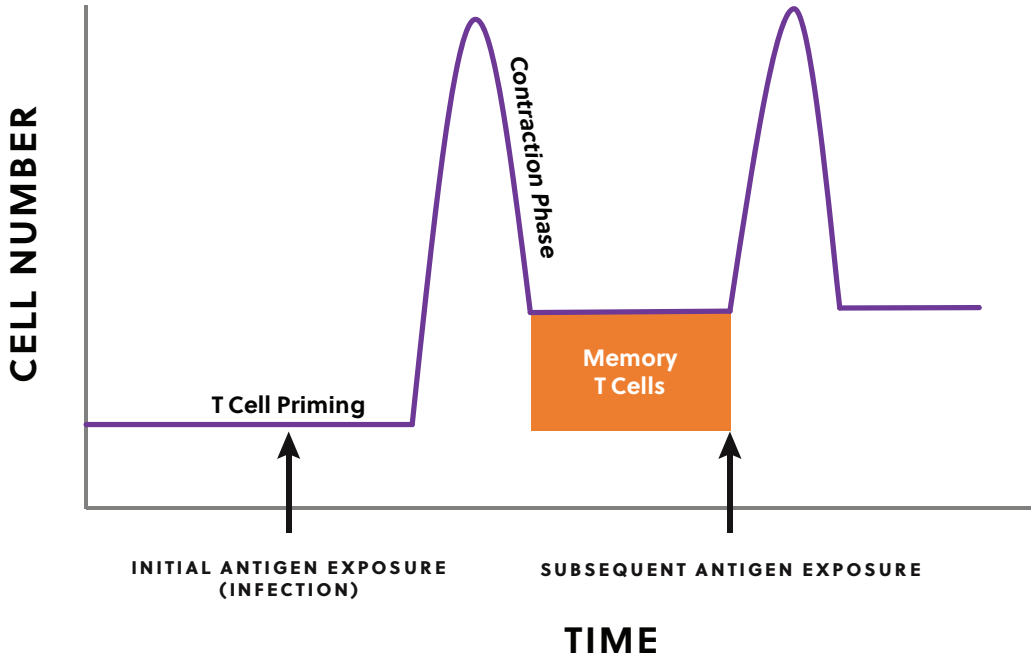

FIGURE 3: ANTIGEN-PRESENTING CELLS PRIME T CELLS, AND MEMORY T CELLS EXPEDITE THE ADAPTIVE IMMUNE RESPONSE

Following infection, antigen-presenting cells are activated and migrate to secondary lymphoid organs, where they present pathogenic peptides to T cells. This T cell priming phase creates a lag between the initial antigen exposure and the T cell response. Activated T cells undergo robust proliferation and clear the infection. After the infection resolves, the contraction phase begins: the majority of these effector T cells undergo apoptosis, but a small percentage become memory cells. Memory cells do not require priming during subsequent antigen exposures, expediting the immune response time.

T cells are a major component of the adaptive immune system and are key mediators of cellular immunity. These cells express T cell receptors (TCRs), which are nongermline-encoded receptors generated by V(D)J recombination that recognize an immense number of molecules. T cells are defined by the TCR chains expressed. In αβ T cells, TCRα and TCRβ chains dimerize to create αβ TCRs, and TCRγ and TCRδ chain heterodimers are present on γδ T cells (**see inset**). αβ T cells primarily localize to secondary lymphoid organs, such as lymph nodes and the spleen, and in contrast to B cells, which directly bind antigens, T cells recognize only small peptide fragments bound to major histocompatibility complex (MHC) molecules (known as human leukocyte antigens [HLAs] in humans, **Table 3**). αβ T cells are further subdivided into CD4+ or CD8+ T cell populations, based

on CD4 or CD8 coreceptor expression, respectively. When TCRs interact with peptide-bound MHC molecules in the presence of costimulatory molecules, αβ T cells are activated, inducing rapid T cell expansion. After infections subside, T cells enter a contraction phase, during which the T cell number is reduced and memory T cells are generated (**Figure 3**). T cell activation can be inhibited by co-inhibitory molecules, such as PD-1 and CTLA-4, and some cancer immunotherapies target these molecules to promote T cell activation and effector function.

γδ T CELLS

γδ T cells, which are mainly found at epithelial surfaces, act at the interface of the adaptive and innate immune systems and are activated through MHC-independent mechanisms. γδ T cells can inhibit or promote tumor progression through IFN-γ or IL-17 secretion, respectively. The antitumorigenic and lytic abilities of γδ T cells make this cell population an attractive candidate for next-generation immunotherapies.

TABLE 3: HUMAN LEUKOCYTE ANTIGEN NOMENCLATURE

Human leukocyte antigen (HLA) genes encode MHC class I and II chains.

	MHC CLASS II	MHC CLASS I
HLA LOCI	HLA A HLA B HLA C	HLA-DP HLA-DQ HLA-DR

CD4+ T Cells

Markers: CD3+ CD4+ **Percent of PBMCs:** 25–55%

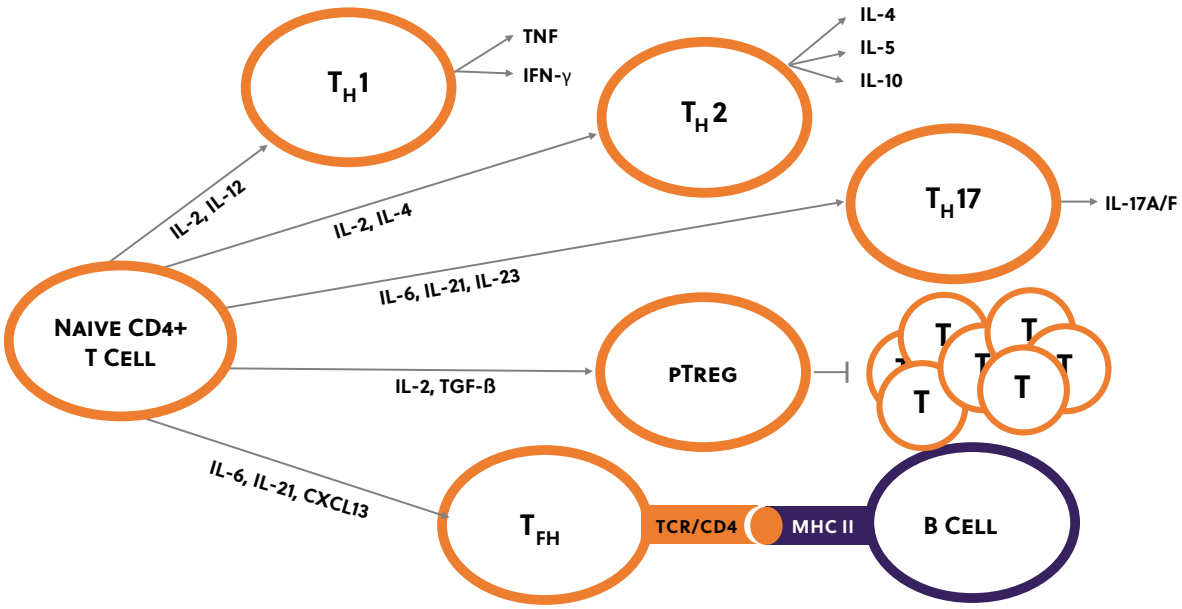

FIGURE 4: CYTOKINE SIGNALING STIMULATES NAÏVE CD4+ T CELL DIFFERENTIATION INTO EFFECTOR CELLS

Cytokines promote CD4+ T cell differentiation into effector T cells. The resulting effector T cells propagate the immune response by secreting cytokines or facilitating other effects.

αβ T cells that express the CD4 coreceptor, referred to as CD4+ T cells or helper T (T_H) cells, recognize short, extracellularly derived peptide fragments bound to MHC class II molecules on antigen-presenting cells (APCs), such as B cells, macrophages, and dendritic cells. The environmental cues (primarily cytokines) present during activation initiate CD4+ T cell differentiation into effector T cells, such as T_H1, T_H2, T_H17, follicular helper (T_{FH}), and regulatory T cells (Tregs), that each have distinct immune functions (**Figure 4**). T_H1 cells fight intracellular pathogens by enhancing CD8+ T cell activity and macrophage activation, promoting cell-mediated immunity. In contrast, T_H2 cells are involved in humoral (antibody-mediated) immunity and promote allergic inflammation. T_H17 cells are responsible for the immune response against extracellular pathogens, but this response can also lead to severe inflammation and autoimmunity. T_{FH} cells are involved in T cell/B cell interactions within the germinal center, which improve B cell antibody affinity for antigens. Finally, Tregs have immunosuppressive functions that are important for controlling autoimmunity (**see inset**).

ROLE IN TUMOR IMMUNOLOGY

The antigen-targeting ability of CD4+ T cells suggests that this cell population should inhibit tumor progression. Indeed, several CD4+ effector T cells have antitumor functions.[1] A cytotoxic CD4+ T cell subset with clear antitumor capabilities has been discovered in peripheral blood, and T_H1 cells exert potent antitumor activity, primarily via IFN-γ secretion, CD8+ T cell activation and expansion, and natural killer (NK) cell and macrophage recruitment. T_{FH} cells may also promote antitumor responses by facilitating the production of neutralizing, antitumor antibodies by B cells.

Reportedly, some CD4+ T cell populations, such as T_H2 and T_H17 cells, have both tumor-promoting and tumor-eradicating capabilities.[1] T_H2 cells can recruit innate immune cells into the tumor microenvironment, which potentially produces antitumor effects. However, in several human cancers, an increased T_H2 cell population is associated with tumor progression. The chronic inflammation often associated with T_H17 cells may also promote tumor progression by enhancing angiogenesis and the secretion of proinflammatory cytokines by tumor cells. In contrast, recent studies have suggested that further differentiated T_H17 cells secrete IFN-γ, inducing antitumor effects. The tumor-suppressive capability of Tregs also plays a powerful role in tumor immunity (**see inset**).

REGULATORY T CELLS (TREGS)

Regulatory T cells (Tregs) are an immunosuppressive subset of CD4+ T cells that originate in the thymus (tTregs) or are induced in the periphery (pTregs). The immunosuppressive activity of these cells counteracts the functions of other effector T cells. Tregs are characterized by high CD25 (i.e., IL-2 receptor α chain) and Foxp3 expression, and these cells promote immunosuppression to prevent host autoimmunity. However, in cancer, Treg-induced immunosuppression enables tumorigenic cells to escape immune detection, promoting tumor growth and progression. Tumor-infiltrating Foxp3+ Tregs have been evaluated as a prognostic factor in cancer, and a meta-analysis revealed that the prognostic value of these Tregs is dependent on tumor site, molecular subtype, and tumor stage.[3]

CD8+ T Cells

Markers: CD3+ CD8+ **Percent of PBMCs:** 10-25%

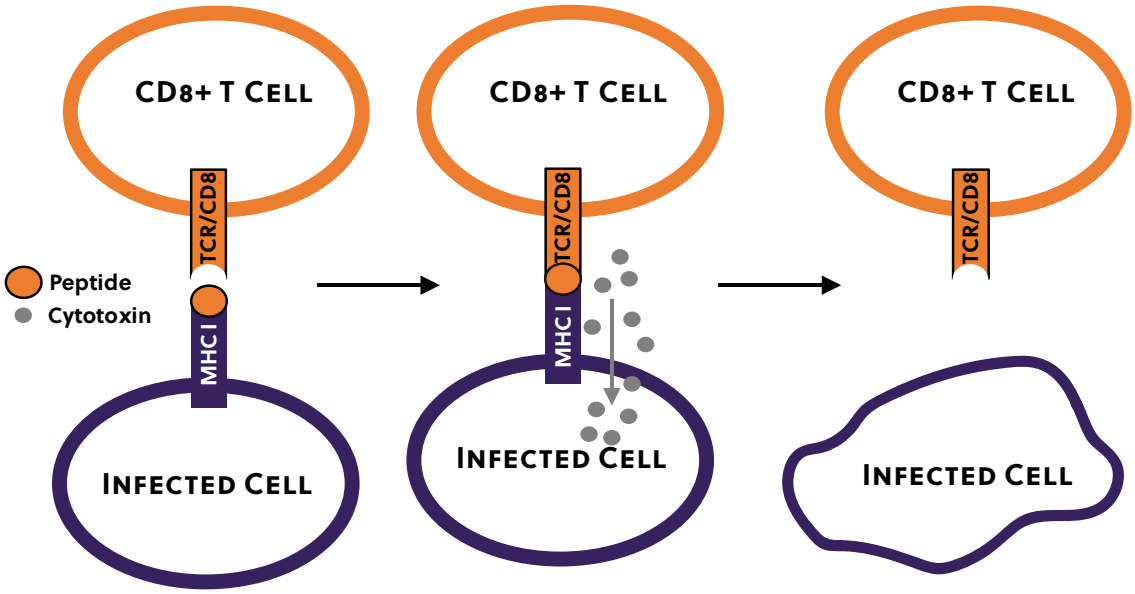

FIGURE 5: CD8+ T CELLS INDUCE CYTOTOXICITY

CD8+ T cell receptors recognize and bind MHC class I-bound antigens on cells. Upon binding, cytotoxic granules are released from the CD8+ T cell, lysing the target cell. This lysis removes virus- and bacteria-infected cells.

αβ T cells that express the CD8 coreceptor are referred to as CD8+ or cytotoxic T cells. In contrast to CD4+ T cells, which recognize MHC class II-bound extracellular protein fragments, CD8+ T cells recognize intracellular protein fragments bound to MHC class I molecules on cell surfaces. While only antigen-presenting cells (APCs) express MHC class II molecules, all cells express MHC class I molecules. The ability of CD8+ T cells to recognize intracellular proteins makes them particularly important in controlling viral and intracellular bacterial infections. Upon activation, CD8+ T cells release cytotoxins (perforin and granzyme B) that initiate apoptosis, directly lysing target cells (**Figure 5**). Through this lysis, virus- and bacteria-infected cells are eradicated. CD8+ T cell-mediated lysis is highly specific, sparing nearby, noninfected cells.

ROLE IN TUMOR IMMUNOLOGY

The cytolytic function of CD8+ T cells is maintained in cancer, where these cells promote tumor cell lysis. CD8+ T cell infiltration is associated with good clinical outcomes in numerous cancers.[4-6] To maximize the antitumor property of CD8+ T cells, tumor cells have been extensively

sequenced to identify tumor-associated antigens (neoantigens) that potentially elicit a CD8+ T cell response.

Several immunotherapies have been developed to enhance CD8+ T cell-mediated activity against tumor cells, including dendritic cell (DC) peptide vaccines. For DC peptide vaccines, DCs are harvested from the patient, exposed to the tumor antigen in vitro, and then infused back into the patient. These DCs express tumor-associated antigens and activate CD8+ T cells to attack and lyse cancer cells. Unfortunately, this tumor-specific, targeted activation of CD8+ T cells is often suppressed by the upregulated expression of inhibitory molecules, such as PD-L1 (a PD-1 ligand), on cancer cells. When PD-L1 on cancer cells interacts with PD-1 on T cells, T cell function is inhibited, allowing the cancer cells to evade T cell-mediated lysis. Several monoclonal antibody drugs, such as Keytruda and Opdiva, target PD-1 to block PD-1/PD-L1 binding, relieving CD8+ T cell inhibition and promoting the CD8+ T cell response.

Chimeric antigen receptor (CAR) T-cell therapy, a recently developed, cell-based immunotherapy, uses the cytolytic activity of CD8+ T cells to target tumor-specific markers.[7] In CAR T-cell therapy, T cells are isolated from a patient and genetically engineered to express a receptor that binds a specific, tumor-associated antigen. After sufficient proliferation, the cells are infused into the patient. CAR T-cell therapies have been developed for advanced and recurrent acute lymphoblastic leukemia and large B cell lymphoma, and this approach is being tested with NK cells and γδ T cells, which also directly lyse target cells.

Naïve T Cells

Naïve CD4+ and CD8+ T cells express the CD45RA surface receptor (**Figure 6**, lower right quadrant) and do not possess effector functions, such as cytokine secretion or cytotoxicity. These cells migrate through the blood, lymphatics, and lymphoid tissues until activated by antigen-presenting cells (APCs). To activate naïve T cells, APCs must simultaneously deliver a specific antigen and the costimulatory molecules CD80 and CD86. Macrophages, B cells, and dendritic cells (DCs) can provide these stimuli, but DCs are the most potent activators. Naïve T cells that interact with an APC undergo robust proliferation and acquire effector functions. When the antigen is cleared, the vast majority of these effector T cells undergo apoptosis, but a small population is converted into memory T cells.

Memory T Cells

Memory CD4+ and CD8+ T cells are generated following antigen encounter and proliferation. Memory T cells have decreased CD45RA expression and increased CD45RO expression (**Figure 6**, upper left quadrant). Memory T cells shorten the adaptive immune response time (**Figure 3**), as these cells remain primed to respond to their respective antigens and have an enhanced proliferative capability. Upon subsequent antigen exposure, memory T cells are rapidly activated and proliferate, expediting the adaptive immune response.

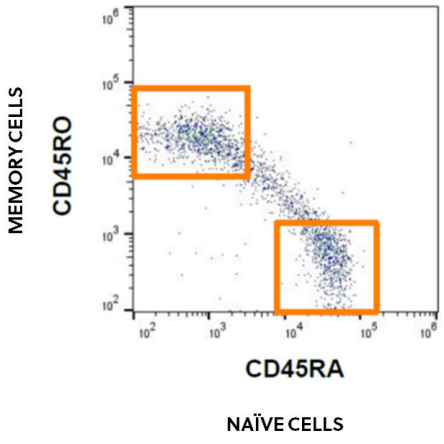

FIGURE 6: NAÏVE AND MEMORY T CELLS EXPRESS UNIQUE CD45 ISOFORMS

Naïve T cells express CD45RA (lower right quadrant). However, when these cells are converted into memory cells (upper left quadrant), CD45RA expression decreases, and CD45RO expression increases. CD45RA/RO expression is analyzed with flow cytometry to distinguish between naïve and memory T cell populations.

Natural Killer (NK) Cells

Markers: CD56+ CD3- **Percent of PBMCs:** 5–20%

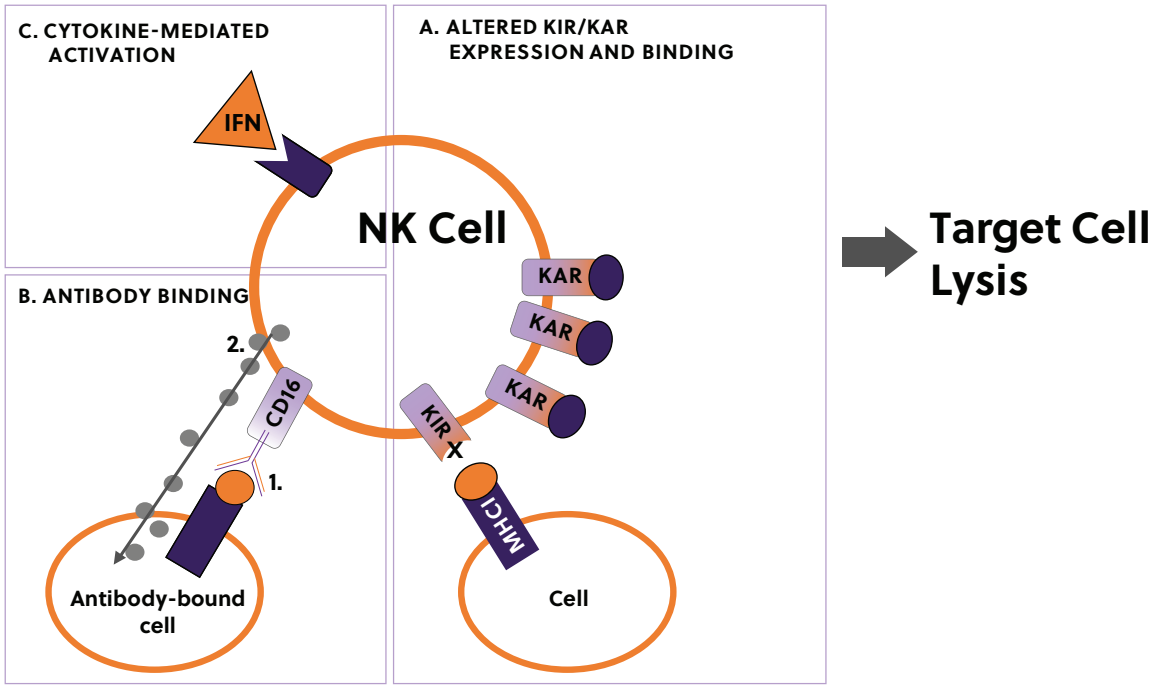

FIGURE 7: NATURAL KILLER CELLS ARE ACTIVATED THROUGH THREE DISTINCT MECHANISMS

A) Natural killer (NK) cells express killer activating receptors (KAR) and killer cell immunoglobulin-like receptors (KIRs) that affect NK cell activation. KIRs bind MHC class I-bound molecules to inhibit NK cell activation, while KAR/ligand binding promotes NK cell activation. B) When NK cell receptors bind antibodies on other cells, NK cells are activated and release cytotoxic granules that lyse the antibody-bound cell through a process called antibody-dependent cell-mediated cytotoxicity. C) NK cells are activated by specific cytokine binding.

Natural killer (NK) cells are a type of lymphocyte with potent cytotoxicity. NK cells are activated by three distinct mechanisms (**Figure 7**). First, NK cells express killer activating receptors (KARs) and killer immunoglobulin-like receptors (KIRs) that promote and inhibit NK cell activation, respectively. KIRs bind MHC class I molecules to inhibit NK cell activity. However, certain cellular stresses (such as a viral infection) upregulate KAR ligand levels and downregulate MHC class I molecules, reducing inhibitory signaling and increasing NK cell activity. Second, NK cells are activated by antibodies bound to target cells and directly lyse the antibody-bound cell through antibody-dependent cell-mediated cytotoxicity (ADCC). Third, NK cells are activated by soluble cytokines, such as interferons (IFNs). After being activated through any of these pathways, NK cells release cytotoxic granules that permeate

target cell membranes to deliver cytotoxic proteins (perforin and granzymes). NK cells also influence the adaptive immune response by interacting with T cells, B cells, and dendritic cells (DCs) and by secreting cytokines and chemokines.

ROLE IN TUMOR IMMUNOLOGY

NK cells were originally considered protective against disease and believed to promote cancer cell lysis and DC activation. The NK group 2D (NKG2D) receptor on NK cells promotes cancer cell death via interactions with ligands (i.e., MICA and MICB) on the tumor surface and is the focus of several preclinical cancer trials. In some cancers, this targeting mechanism appears to be inhibited, but monoclonal antibodies are being used to circumvent this unfortunate dilemma.[8]

NATURAL KILLER T (NKT) CELLS

Markers: CD56+ CD3+

Natural killer T (NKT) cells express CD56 and αβ TCR, which are the prototypical markers of NK and T cells, respectively (**Figure 8**). Although NKT cells express TCRs, these TCRs differ from those on CD4+ or CD8+ T cells: NKT cell TCRs are invariant and thus have a limited ability to recognize ligands. Instead of recognizing MHC-bound molecules, TCRs on NKT cells interact with CD1d-bound lipid and glycolipid ligands. Notably, NKT cells can activate NK cells by secreting IFN.

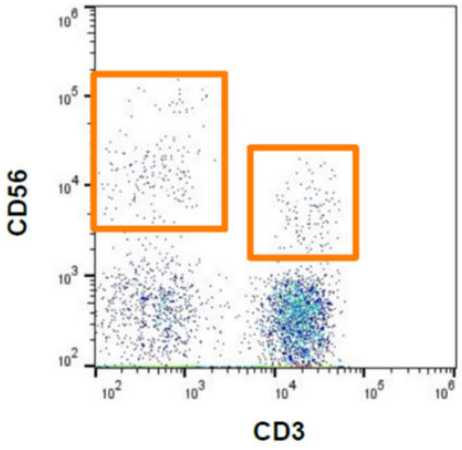

FIGURE 8: NATURAL KILLER CELLS AND NATURAL KILLER T CELLS HAVE UNIQUE CD56/CD3 EXPRESSION PATTERNS

Natural killer (NK) cells (CD56+ CD3-, upper left quadrant) and NK T cells (CD56+ CD3+, upper right quadrant) were identified by flow cytometric analysis.

Monocytes

Human Markers: CD14+ **Percent of PBMCs:** 10–25%

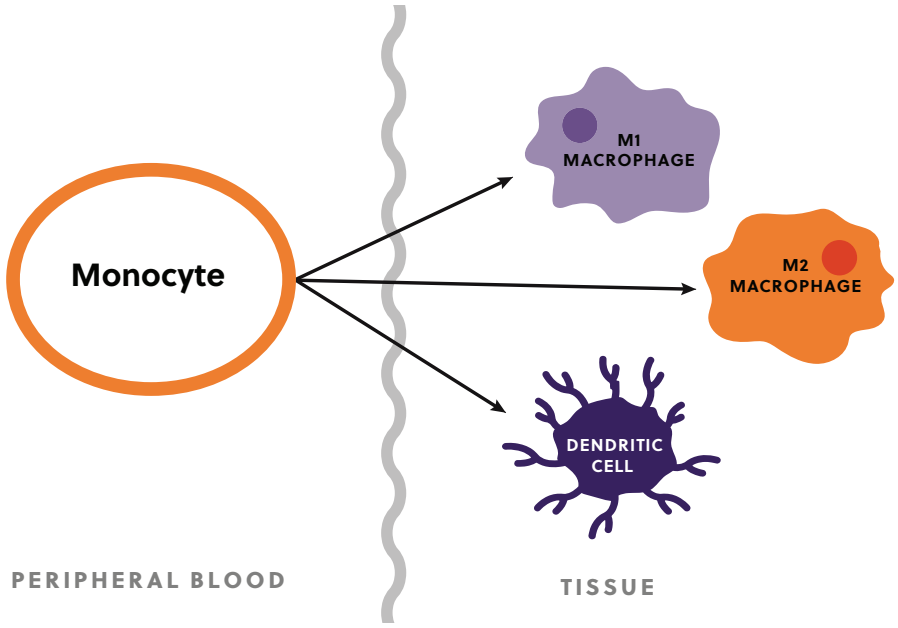

FIGURE 9: MONOCYTES DIFFERENTIATE IN TISSUE

Monocytes in peripheral blood extravasate into tissues and differentiate into macrophages (M1 or M2) or dendritic cells.

Monocytes are immature cells that can differentiate into multiple antigen-presenting cell (APC) populations, primarily macrophages or dendritic cells (DCs, Figure 9), depending on the extracellular signal present. Macrophages mature from monocytes that have extravasated from blood into tissue. This cell population is subdivided into proinflammatory M1 or anti-inflammatory M2 macrophages and mediates the innate immune system through phagocytosis, the process by which dead cells, cellular debris, and microbes are engulfed. The cell antigens internalized through phagocytosis are recycled and presented, bound to MHC class II molecules, on the macrophage cell surface. Monocytes can also differentiate into DCs, which are potent APCs that interact with T cells. In addition to phagocytosis, monocytes, macrophages, and DCs secrete cytokines, influencing the overall immune response.

ROLE IN TUMOR IMMUNOLOGY

The ability of monocytes to differentiate into diverse APCs suggests that this cell population may have multiple effects on tumor growth. Indeed, monocytes both inhibit and stimulate the T cell response to affect tumor progression.

Classically, proinflammatory M1 macrophages possess antitumor properties, and anti-inflammatory M2 macrophages promote tumor growth. However, recent studies have revealed that tumor-associated macrophages (TAMs) exist on a spectrum of activation and differentiation and are not defined by a dichotomous pro- or anti-inflammatory state.[9] TAMs are often associated with poor prognosis due to the secretion of immunosuppressive cytokines and the expression of inhibitory molecules, such as PD-L1.[10,11]

For information on the roles of DCs in tumor immunity, please refer to page 17.

Dendritic Cells

Percent of PBMCs: <5%

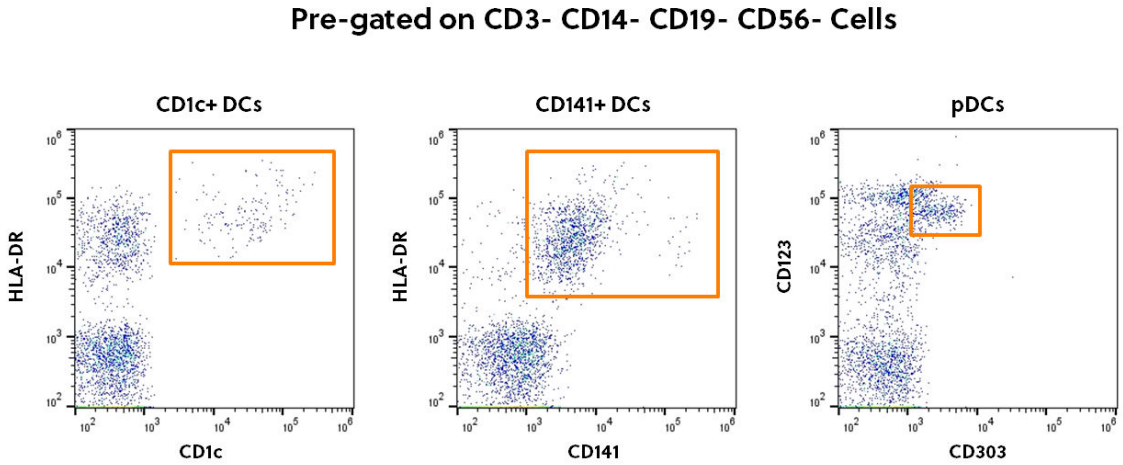

FIGURE 10: THREE TYPES OF DENDRITIC CELLS EXIST IN PERIPHERAL BLOOD

Lineage-negative cells [CD3- CD19- CD14- CD56-] were analyzed by flow cytometry, and CD1c+ dendritic cells (DCs), CD141+ DCs, and CD123+ CD303+ plasmacytoid DCs were identified.

Three types of dendritic cells (DCs) exist in blood: CD1c+ and CD141+ myeloid DCs and CD303+ plasmacytoid DCs (pDCs) (**Figure 10**). These DCs are relatively immature, with underdeveloped dendrites; however, each cell type performs a unique, essential function. CD1c+ DCs recruit immune cells via cytokine secretion. CD141+ DCs are involved in cross-presentation, the process by which exogenous MHC class II-bound antigens are internalized and presented on the cell surface bound to MHC class I molecules. Cross-presentation allows DCs to activate the CD8+ T cell response without prior pathogen infection. Viral infections activate pDCs, which secrete high levels of type-I IFNs. As antigen-presenting cells (APCs), activated pDCs upregulate MHC class I and II molecule expression and increase costimulatory molecule levels to activate T cells; thus, DCs link the adaptive and innate immune system.

ROLES IN TUMOR IMMUNOLOGY

The potent ability of DCs to activate and enhance T cell responses is crucial to the initiation of antitumor immune responses. Activated DCs can engulf and cross-present tumor-associated antigens to CD8+ T cells, stimulating a CD8+ T cell response. Unfortunately, DC function is often dysregulated in the immunosuppressive tumor microenvironment, and pDC function may be specifically disrupted, resulting in the inhibition of CD4+ and CD8+ T cell

proliferation and the activation of regulatory T cells (Tregs).[12]

DC vaccines that initiate antitumor immune responses have been successfully developed and applied. In this process, DC progenitors are isolated from a patient, and then, DC differentiation is induced in the presence of cancer cells or cancer-related antigens. These primed cells are injected back into the patient, where they activate CD8+ T cells to attack and eradicate cancer cells.

Stem Cells

Human Markers: CD34+ **Percent of PBMCs:** <1%

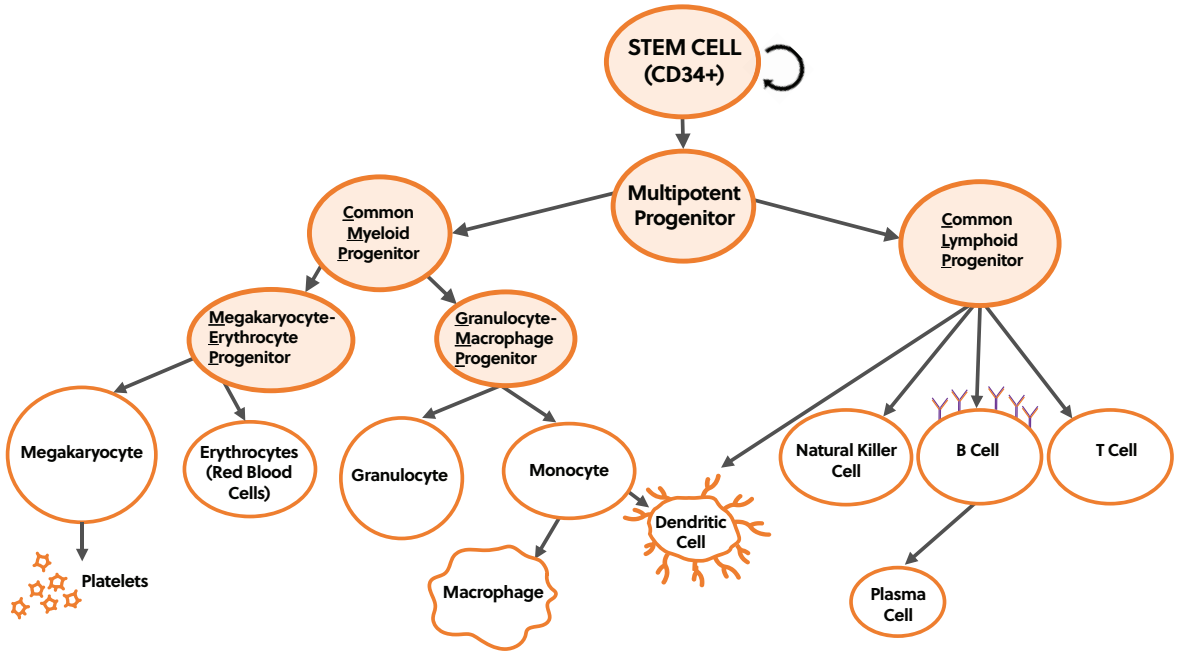

FIGURE 11. HEMATOPOIETIC STEM CELLS DIFFERENTIATE INTO PERIPHERAL BLOOD CELLS

CD34+ stem cells have the capacity to differentiate into all the downstream hematopoietic cell types, including anucleate red blood cells/platelets, mononuclear lymphocytes/monocytes, and multilobed granulocytes.

CD34+ stem cells have the capacity to differentiate into all hematopoietic cell types (**Figure 11**). Bone marrow contains the greatest number of CD34+ stem cells, but a small population of undifferentiated CD34+ stem cells is present in peripheral blood. When the appropriate external environmental factors are encountered, stem cells differentiate into progenitor cells, which then mature into blood cells. These external factors include soluble factors, such as cytokines and growth factors, and surface receptor/receptor interactions.

Appendix I: Current Immunotherapies

LYMPHOCYTE-DIRECTED THERAPIES

Lymphocyte-directed therapies use antibodies to label dysregulated, cancerous lymphocytes, which are then attacked and eradicated by innate immune cells (i.e., macrophages and natural killer cells).

Alemtuzumab (Lemtrada®) targets the CD52 antigen on lymphocytes, causing immune cells to attack and destroy lymphocytes.

Indications: Chronic lymphocytic leukemia

Brentuximab vedotin (Adcetris®) delivers chemotherapy to lymphocytes by targeting CD30.

Indications: Hodgkin's lymphoma and anaplastic large cell lymphoma

Denileukin diftitox (Ontak®) is an IL-2-based drug that delivers diphtheria toxin to T cells by targeting the CD25 antigen.

Indications: Cutaneous T cell lymphoma

Ibritumomab tiuxetan (Zevalin®) is a monoclonal antibody that targets the CD20 antigen expressed on B cells. This antibody delivers radioimmunotherapy to B cells.

Indications: Non-Hodgkin's lymphomas

T CELL-DIRECTED THERAPIES

T cell-directed therapies primarily attack cancer cells by activating the lytic ability of CD8+ T cells.

Blinatumomab (Blincyto®) binds CD3 on T cells and CD19 on cancer cells to bring these two cells into proximity, expediting T cell-induced cancer cell lysis.

Indications: Refractory acute lymphoblastic leukemia

Chimeric antigen receptor (CAR) T-cell therapy genetically engineers patient T cells to express a chimeric antigen receptor against a specific cancer cell ligand. After in vitro culturing, these cells are reinfused into the patient, where they target cancer cells.

Indications: Advanced/recurrent acute lymphoblastic leukemia, advanced/recurrent large B cell lymphoma

Pembrolizumab and nivolumab (Keytruda® and Opdivo®, respectively) are two monoclonal antibodies that target PD-1 to promote T cell activation.

Indications: Melanoma, non-small cell lung cancer, kidney cancer, bladder cancer, head and neck cancers, Hodgkin's lymphoma

Atezolizumab, avelumab, and durvalumab (Tecentriq®, Bavencio®, and Imfinzi®, respectively) target the PD-1 ligand, PD-L1, to promote T cell activation.

Indications: Bladder cancer, non-small cell lung cancer, Merkel cell carcinoma

Ipilimumab (Yervoy®) is a monoclonal antibody that targets CTLA-4 to promote T cell activation.

Indications: Melanoma

Sipuleucel-T (Provenge®) is a therapy that uses in vitro techniques to induce patient immune cell differentiation into dendritic cells, which promote T cell-mediated cancer cell lysis.

Indications: Advanced prostate cancer

GENERAL IMMUNE-BOOSTING THERAPIES

Immune-boosting therapies promote the entire immune system through known or unknown mechanisms.

Synthetic IL-2 treatment promotes immune cell proliferation.

Indications: Renal cancer, metastatic melanoma

Synthetic IFN-α treatment enhances immune cell anticancer capacity and may directly decrease tumor and blood vessel growth.

Indications: Hairy cell leukemia, chronic myelogenous leukemia, follicular non-Hodgkin's lymphoma, cutaneous T cell lymphoma

Lenalidomide, pomalidomide, and *thalidomide* (Revlimid®, Pomalyst®, and Thalomid®, respectively) enhance the immune response against cancer through an unknown mechanism.

Indications: Multiple myeloma

Bacille Calmette-Guérin is a vaccine used to activate the immune response in tissues.

Indications: Early stage bladder cancer, melanoma skin cancers

Imiquimod (many brands) is topically applied to induce a local immune response.

Indications: Early stage skin cancer

Appendix II: Additional Information

ADAPTIVE IMMUNITY

Adaptive immunity, also called acquired immunity, is initiated in the presence of perceived foreign matter. The key cell types of the adaptive immune system, T cells and B cells, have highly diverse surface receptors that specifically recognize target antigens. Additionally, the adaptive immune system generates immune memory cells, which are activated quickly upon reinfection (**Figure 3**).

INNATE IMMUNITY

Innate immunity is the initial defense against foreign pathogens. Cells of the innate immune system are activated by soluble factors, such as cytokines, or by highly conserved pathogenic factors, such as lipopolysaccharide (LPS), double stranded RNA (dsRNA), or flagellin. The innate immune system also activates the adaptive immune system.

LYMPHOCYTES

In healthy humans, lymphocytes, namely B cells, T cells, and natural killer (NK) cells, are present within both the blood and lymphatic system. These cells compose 20–40% of the white blood cell population. B cells and T cells aid adaptive immunity, while NK cells are involved in innate immunity.

CYTOKINES

Cytokines, including interleukins (ILs), interferons (IFNs), and tumor necrosis factors (TNFs), are key secretory proteins that mediate immune signaling and immune cell differentiation. Each immune cell subset is stimulated by distinct extracellular cytokines and secretes a unique cytokine profile. These responses are often altered during disease. When working with a specific immune cell population, it is important for scientists to consider which cytokines are secreted by that cell type, which cytokines influence that cell's behavior, and which disease conditions might alter cytokine levels to influence the immune response.

CLUSTER OF DIFFERENTIATION (CD) NOMENCLATURE

Cluster of differentiation (CD) proteins (i.e., CD markers) are molecules on the cell surface. This nomenclature assigns a unique number to each receptor, enabling each cell type to be defined according to the presence (+) and/or absence (-) of receptors. For example, CD4+ T cells express the CD4 coreceptor but not CD8 (CD4+ CD8-); B cells express both CD19 and CD20 (CD19+ CD20+); and natural killer cells express CD56 but not CD3 (CD56+ CD3-). CD markers are easily identified with immunophenotyping techniques, such as immunohistochemistry

and flow cytometry, enabling cell populations to be detected and isolated from blood samples for further study.

MAJOR HISTOCOMPATIBILITY COMPLEX (MHC) MOLECULES

Two classes (class I and II) of major histocompatibility complex (MHC) molecules are expressed on cells. Intracellular peptides are bound to MHC class I molecules and then expressed on the cell surface. All cells express MHC class I molecules, but most peptides bound to MHC class I molecules are not perceived as a threat and thus do not elicit an immune response. However, if foreign peptides, such as pathogenic or mutated protein fragments, are presented, a CD8+ T cell response is activated.

In contrast, MHC class II molecules are present on antigen-presenting cells (APCs) only. APCs internalize and digest extracellular proteins into peptides. Then, the peptides are attached to MHC class II molecules and presented on the cell surface, where they elicit a CD4+ T cell response.

Appendix III: Glossary of Terms

αβ T cell – A T cell expressing αβ TCR chains; these cells recognize small pathogen-derived fragments bound to MHC molecules, activating T cell expansion

γδ T cell – A T cell expressing TCRs with γδ chains; this cell type recognizes intact proteins

Acquired immunity – *See adaptive immunity*

Adaptive immunity – Highly specific antibody- and cell-mediated immunity that develops following the initial antigen exposure; also called "acquired" immunity

Affinity maturation – The process that improves B cell receptor (BCR) affinity for a specific antigen

Antibody-dependent cell-mediated cytotoxicity – The process in which an immune system effector cell (i.e., natural killer cell) is activated by the antibodies bound to a target cell's antigens and then lyses the antibody-bound cell

Antigen-presenting cell (APC) – A cell that expresses antigen on its surface (e.g., dendritic cells, macrophages, and B cells) and can activate CD4+ and CD8+ T cells

Antigen – A molecule that stimulates the adaptive immune system

Anucleated cell – A cell without a nucleus (i.e., erythrocytes/red blood cells and thrombocytes/platelets)

B cell receptor (BCR) – An antigen-binding receptor on the B cell surface

Cell-mediated immunity – A T cell-mediated adaptive immune response

Class switch recombination – The process by which the BCR immunoglobulin isotype changes, thereby altering the activity triggered upon antigen binding

Cross-presentation – The process by which MHC class II-bound antigens are internalized by CD141+ DCs and presented on the DC surface through the MHC class I pathway, triggering a CD8+ T cell response

Cytokines – Small, secreted proteins that can elicit cell-specific responses

Cytotoxic T cells – CD8+ αβ T cells that release cytotoxins to promote the apoptosis of target cells

Dendritic cell (DC) – An antigen-presenting cell that activates the adaptive immune system

Flow cytometry – A laboratory technique that uses specific antibodies to identify unique cell populations

Germinal center – A site in secondary lymphoid organs where B cells interact with T cells to promote affinity maturation and class switch recombination

Granulocyte – A white blood cell with a multilobed nucleus

Helper T (T_H) cell – A CD4+ αβ T cell

Highly conserved factor – Nucleic acid or peptide sequence that has been maintained throughout the evolutionary process and thus exists in many species

Humoral immunity – The adaptive immune response mediated by B cells and antibodies

Immunoglobulin A (IgA) – An antibody isotype that protects against inhaled or ingested pathogens

Immunoglobulin D (IgD) – An antibody isotype that is coexpressed with IgM and secreted from B cells in the upper respiratory mucosa

Immunoglobulin E (IgE) – An antibody isotype that is located on mast cell surfaces and involved in allergic reactions

Immunoglobulin G (IgG) – The most common antibody isotype in human serum and the main potentiator of humoral immunity

Immunoglobulin isotype – The immunoglobulin class, which is characterized by the heavy chain (A/α, D/δ, E/ε, G/γ, M/μ)

Immunoglobulin M (IgM) – The first and largest antibody isotype produced; its presence indicates recent infection

Innate immunity – The nonspecific, initial immune response against highly conversed pathogenic stimuli

Interferon (IFN) – A family of cytokines involved in innate and acquired immunity

Interleukin (IL) – A family of cytokines involved in innate and acquired immunity

Lymphocyte – A small, nucleated white blood cell that circulates through the blood and lymphatics and is involved in the immune response

Major histocompatibility complex (MHC) – Molecules on the cell surface that present host- and pathogen-derived peptide fragments; all cells express MHC class I molecules, but only antigen-presenting cells express MHC class II molecules

Memory T cell – A previously activated CD4+ or CD8+ T cell that protects against subsequent exposures to familiar pathogens

Monocyte – A type of white blood cell involved in innate immunity that can differentiate into macrophages or dendritic cells

Naïve T cell – A CD4+ or CD8+ T cell that has not been activated

Natural killer T (NKT) cell – A T cell that also shares natural killer cell properties

Peripheral blood mononuclear cell (PBMC) – A peripheral blood cell with a single, rounded nucleus

Phagocytosis – The cellular internalization of large particles

Plasmacytoid dendritic cell (pDC) – A cell with plasma cell-like morphology that accumulates in the lymph nodes and at sites of inflammation

Programmed cell death – Apoptosis-mediated cell death

Regulatory T cell (Treg) – A CD4+ T cell that suppresses the immune response

Stem cell – An undifferentiated CD34+ cell that possesses potent proliferation ability and can differentiate into all downstream hematopoietic cell types

T cell receptor (TCR) – A receptor on T cells that recognizes short MHC class I- or class II-bound peptides; the chains (α/β or γ/δ) that compose the TCR determine the T cell type (i.e., αβ or γδ T cell)

Tumor necrosis factor (TNF) – A proinflammatory cytokine involved in immunity

V(D)J recombination – The process by which variable (V), diversity (D), and joining (J) gene segments are assembled to generate BCRs and TCRs with unique binding specificity

White blood cell – A cell with a rounded or multilobed nuclei involved in immunity (i.e., granulocytes, monocytes, and lymphocytes)

Appendix IV: Abbreviations

APC – Antigen-presenting cell

BCR – B cell receptor

CD – Cluster of differentiation

DC – Dendritic cell

IFN – Interferon

Ig – Immunoglobulin

IL – Interleukin

NK cell – Natural killer cell

NKT cell – Natural killer T cell

pDC – Plasmacytoid dendritic cell

PBMC – Peripheral blood mononuclear cell

TCR – T cell receptor

T_{FH} cell – Follicular helper T cell

T_H cell – Helper T cell

Treg – Regulatory T cell

References

1. Kim H-J, Cantor H. CD4 T-cell subsets and tumor immunity: the helpful and the not-so-helpful. *Cancer Immunol Res*. 2014;2(2):91-98. doi:10.1158/2326-6066.CIR-13-0216

2. Tsou P, Katayama H, Ostrin EJ, Hanash SM. The emerging role of B cells in tumor immunity. *Cancer Res*. 2016;76(19):5597-5601. doi:10.1158/0008-5472.CAN-16-0431

3. Shang B, Liu Y, Jiang S, Liu Y. Prognostic value of tumor-infiltrating FoxP3+ regulatory T cells in cancers: a systematic review and meta-analysis. *Sci Rep*. 2015;5(1):15179. doi:10.1038/srep15179

4. Barnes TA, Amir E. HYPE or HOPE: the prognostic value of infiltrating immune cells in cancer. *Br J Cancer*. 2017;117(4):451-460. doi:10.1038/bjc.2017.220

5. Hwang W-T, Adams SF, Tahirovic E, Hagemann IS, Coukos G. Prognostic significance of tumor-infiltrating T cells in ovarian cancer: a meta-analysis. *Gynecol Oncol*. 2012;124(2):192-198. doi:10.1016/j.ygyno.2011.09.039

6. Ziai J, Gilbert HN, Foreman O, et al. CD8+ T cell infiltration in breast and colon cancer: A histologic and statistical analysis. *PLoS One*. 2018;13(1):e0190158. doi:10.1371/journal.pone.0190158

7. June CH, O'Connor RS, Kawalekar OU, Ghassemi S, Milone MC. CAR T cell immunotherapy for human cancer. *Science*. 2018;359(6382):1361-1365. doi:10.1126/science.aar6711

8. Cerwenka A, Lanier LL. Natural killers join the fight against cancer. *Science*. 2018;359(6383):1460-1461. doi:10.1126/science.aat2184

9. Mosser DM, Edwards JP. Exploring the full spectrum of macrophage activation. *Nat Rev Immunol.* 2008;8(12):958-969. doi:10.1038/nri2448

10. Kuang D-M, Zhao Q, Peng C, et al. Activated monocytes in peritumoral stroma of hepatocellular carcinoma foster immune privilege and disease progression through PD-L1. *J Exp Med*. 2009;206(6):1327-1337. doi:10.1084/jem.20082173

11. Zhang Q, Liu L, Gong C, et al. Prognostic significance of tumor-associated macrophages in solid tumor: a meta-analysis of the literature. *PLoS One*. 2012;7(12):e50946. doi:10.1371/journal.pone.0050946

12. Li S, Wu J, Zhu S, Liu Y-J, Chen J. Disease-associated plasmacytoid dendritic cells. *Front Immunol*. 2017;8:1268. doi:10.3389/fimmu.2017.01268

Notes

Discovery Life Sciences is the life science industry's premier provider of research services and biospecimen solutions. For more information about Discovery Life Sciences, or to inquire about services or biospecimens, please visit **www.dls.com/biopharma**

AUTHORS:

Shawn P. Fahl PhD

Rachel Lane PhD, RD

DISCOVERY

LIFE SCIENCES

Science at your Service.™