IMMUNOLOGY

Guide to Insight into the Diversity of Cells and Signaling Pathways

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PURPOSE AND SCOPE

What to Expect?

Welcome to this Immunology Booklet, a document that helps scientists and researchers appreciate and navigate the diversity of the cell types and molecular pathways associated with immunity and the unfolding of immune responses. We hope the visuals provided in this document will help shed light on and clarify otherwise complex mechanisms.

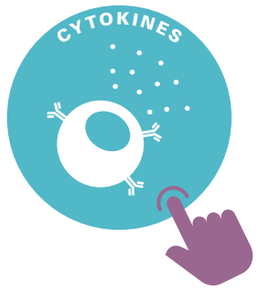
This document is organized around the distinction between innate and adaptive immunity. A short introduction visually summarizes the immune response and cell types it involves, before clarifying lineage notions and distinctions between the cells. It is followed by a section dedicated to innate immunity. Details are given about the key strategies, cell types, and receptors involved. The last section covers adaptive immunity in a similar way.

The collection of molecular pathways presented in the document was prepared based on authentic and highly regarded articles and journals. Numbers in brackets throughout the booklet correlate with the references used, and all pathways have been curated for scientific knowledge and accuracy by Cisbio's scientific team.

Why this Guide?

Inflammation mechanisms are a shared and central part of numerous pathologies including oncology disorders, autoimmunity, virology, neurodegenerative diseases, and metabolic disorders such as diabetes or NAFLD. Additionally, cytokines and chemokines are the key molecules of immunity and are both responsible for and regulated by complex intracellular signaling pathways involving wide arrays of phosphoproteins, mediators, and transcription factors.

This booklet was designed to provide a common ground of knowledge for Cisbio's Inflammation and Immunology content universe, which features a collection of specialized documents dedicated to its different areas and is being constantly expanded. To get an insight into the pathways and processes of specific disorders we recommend looking at our dedicated booklets which currently feature Diabetes and NAFLD. On the other hand, if your interests are more practical and your research involves cytokines or cell signaling, we have written two specialized guides covering the expertise of Cisbio's scientists in assay development and performance.



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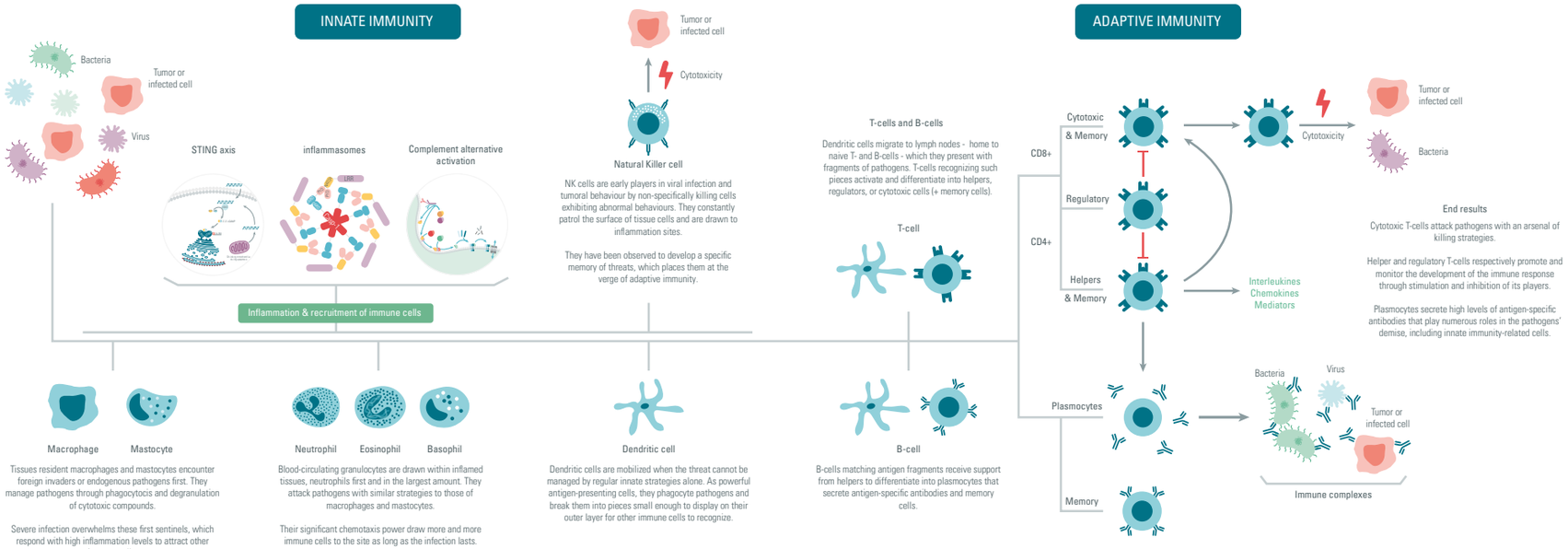
Immunity Overview



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Cellular Lineage

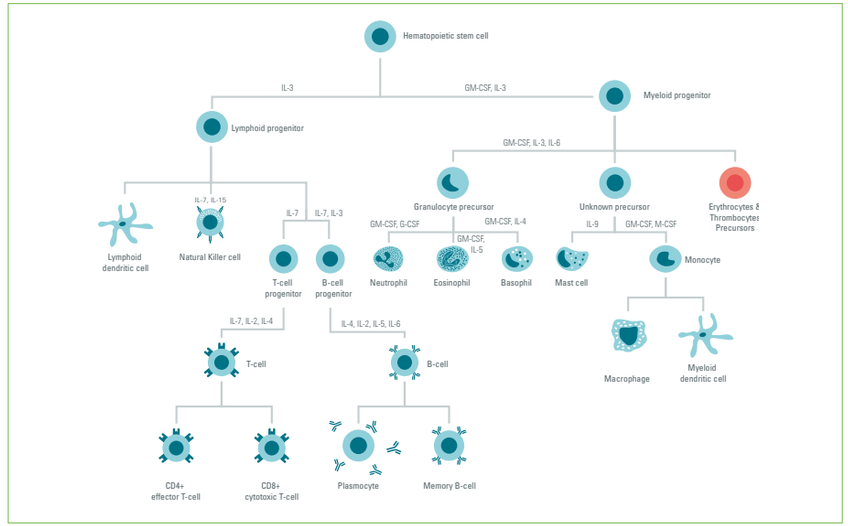
Immune cell types, also called leukocytes or white blood cells, are one of the three blood cell families, along with erythrocytes (red blood cells) and thrombocytes (platelets).They are all differentiated and specialized descendants of the hematopoietic stem cells found in bone marrow.

Leukocytes are usually split into two great cell lines dependending on their oligopotent progenitors, which are either lymphoid or myeloid and result from the commitment of the multipotent hematopoietic stem cells to a differentiation axis. Myeloid progenitors are responsible for the generation of most cells ensuring innate immunity related roles. These include the different granulocytes but also tissue-resident macrophages and mast cells, as well as dendritic cells. On the other hand, lymphoid progenitors originate from the lymphoid cells responsible for adaptive immunity, including B and T cells and the partially innate and adaptive NK cells. It is also understood they can result in dendritic cells of lymphoid lineage.

The polarization and commitment of progenitors and precursors to either line and specialized cell type are processes regulated by transcription factors, whose activity is itself monitored by the signaling and inflammatory environment of cells, especially cytokines.

As cells progress in the two lines, their ability to self-renew and differentiate is narrowed while their number and expression of functionally specific molecules increase. Multipotent uncommited hematopoietic stem cells therefore evolve into oligopotent committed precursors and specialized functional immune cells (1) (2) (3) (4).

In cell-based immunology-related studies, experiments often focus on a subset of leucocytes designated as Peripheral Blood Mononuclear Cells (PBMC). These consist of all peripheral blood cells exhibiting a single round-shaped nucleus. Consequently they include B-cells, T-cells, NK cells, and monocytes, while the multi-lobed granulocytes and nucleus-deprived erythrocytes and platelets are excluded.



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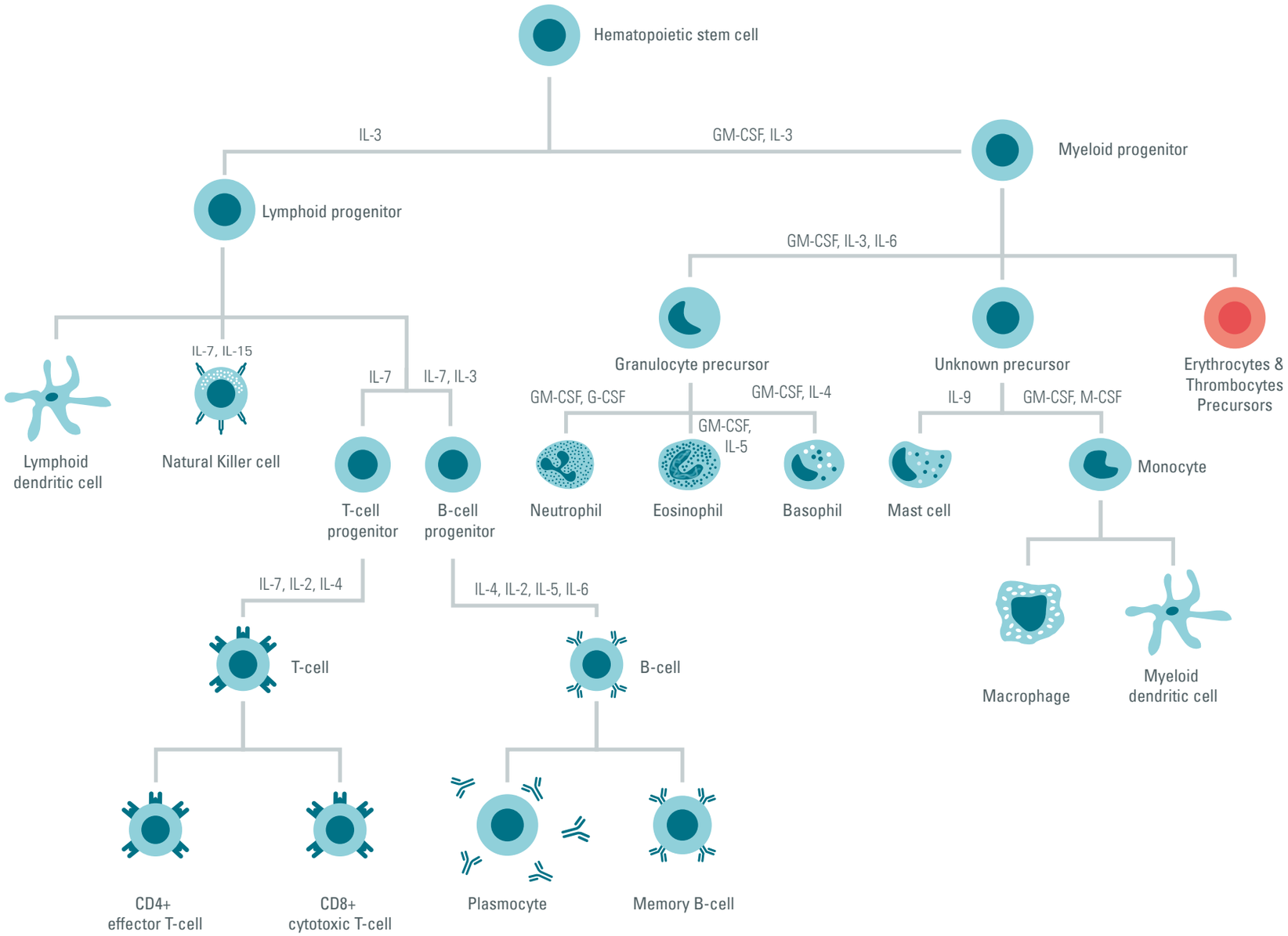

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INNATE IMMUNITY

Key Elements

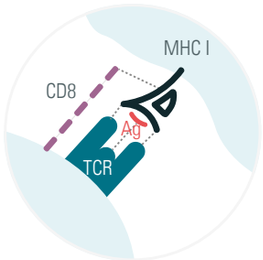
MHC (MAJOR HISTOCOMPATIBILITY COMPLEX)

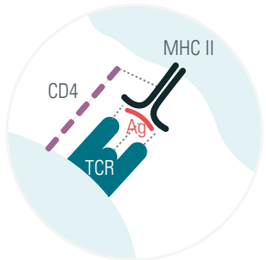
MHCs are transmembrane heterodimeric complexes expressed on all cells, apart from a few exceptions which play a central role in the discrimination between self and foreign antigens. The MHC profile of an organism is unique and does not exist outside of it, making it the standard by which histocompatibility and incompatibility are measured. MHCs exist in two types, depending on the cells, and have antigen presentation properties which involve them in the development of humoral and cell-mediated immunity.

Type I major histocompatibility complexes

MHC I are constitutively expressed by all cells except for red blood cells and special tissues such as the cornea. They are responsible for ensuring the normal state of the cells which carry them by presenting endogenous peptides, and they interact with TCRs and the CD8 of CD8+ T-cells. They tend to become overexpressed by infected, damaged, or tumorous cells, thereby increasing the likelihood of such cells to present antigenic peptides and be removed by CD8+ T-cells.

MHC I are made up of a 3-domain alpha microglobulin chain associated with a beta microglobulin chain to form a closed cavity encapsulating antigenic peptides of 8 to 10 amino acids.

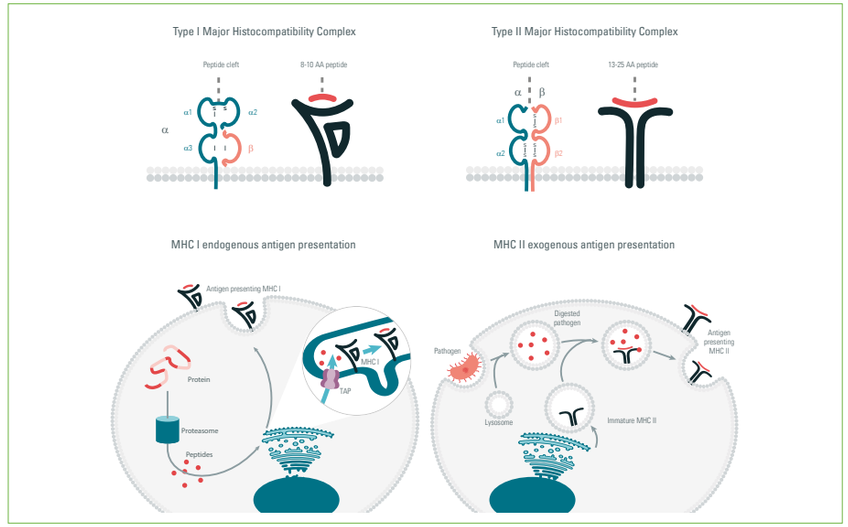




Type II major histocompatibility complexes

MHC II are not carried by all cell types, and are solely constitutively expressed by antigen presenting cells (dendritic cells, monocytes, macrophages, and mast cells) and epithelial thymus cells. MHC II expression can however be induced in other cell types by IFN-γ (5). They are specialized in the presentation of exogenous antigens to CD4+ T-cells, which bind them to their TCRs and CD4.

MHC II are made up of 2-domain alpha and beta microglobulin chains (one of each) associated to form an open cavity in which antigenic peptides can be attached via hydrogen bonds. They present larger antigens than MHC I, ranging from 13 to 25 amino acids (6) (7) (8) (9) (10).



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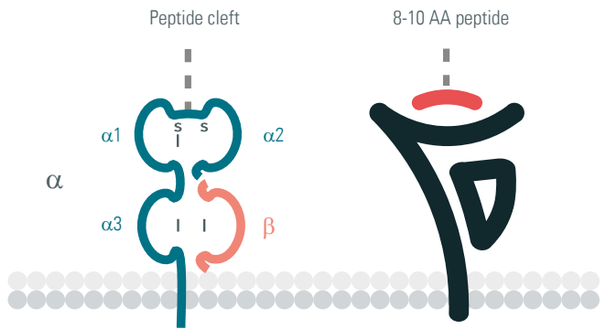
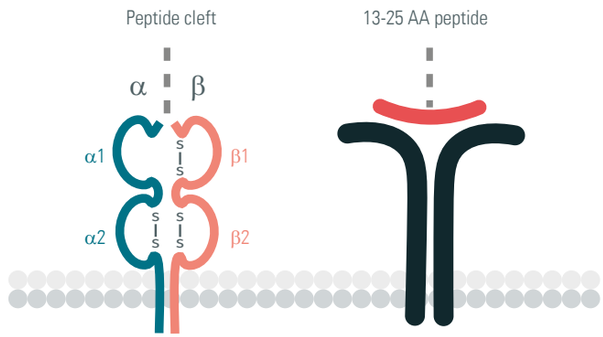
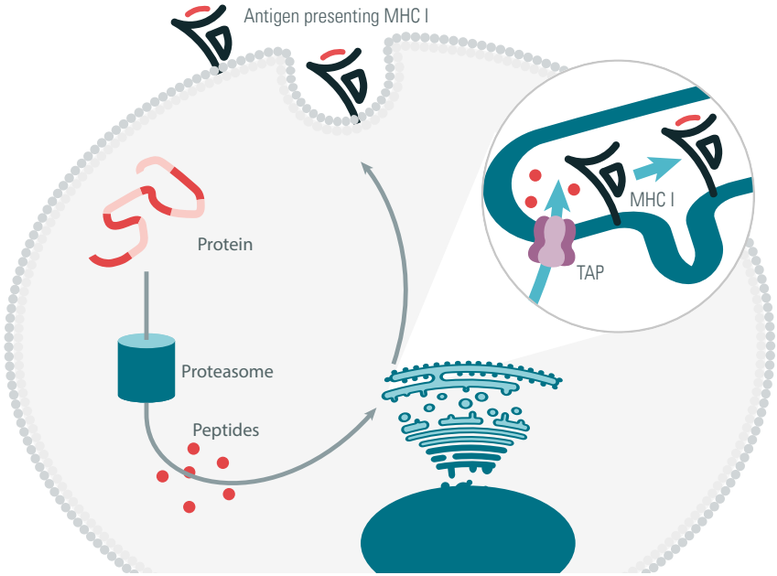
Type I Major Histocompatibility Complex



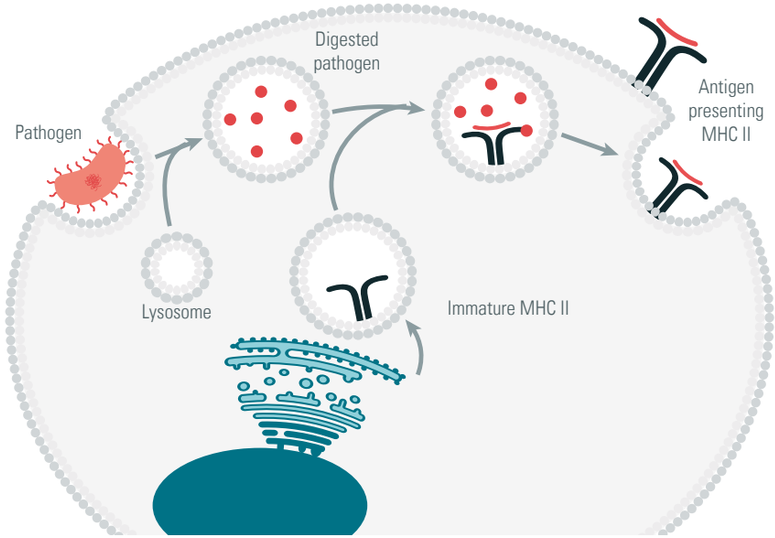
Type II Major Histocompatibility Complex



MHC I endogenous antigen presentation



MHC II exogenous antigen presentation



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INNATE IMMUNITY

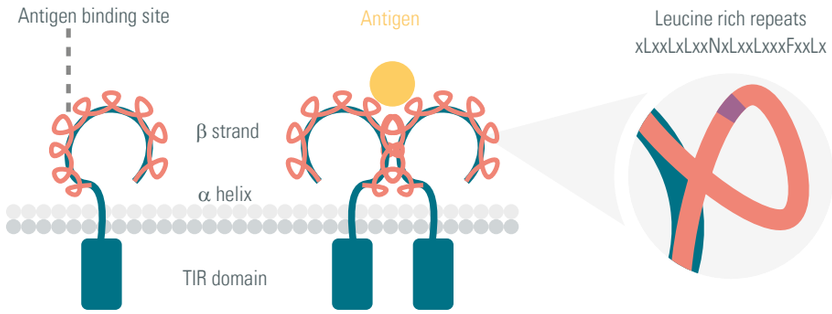
Key Elements

TOLL-LIKE RECEPTORS

Toll-like receptors (TLRs) are a family of evolutionarily conserved pattern recognition receptors (PRRs) that are part of innate immunity. They contribute to the first line of defense against foreign pathogens, and are expressed not only by most immune cell types including macrophages, monocytes, natural killers, T-cells, B-cells, mast cells, and dendritic cells, but also by some non-immune cells such as epithelium or endothelium cells and fibroblasts (11). They play a role of innate antigen receptors by recognizing PAMPs (pathogen-associated molecular patterns) that are specific to micro-organisms. The most commonly recognized among these patterns include molecules that are usually entirely foreign to the organism, such as lipids and proteins from bacterial walls, or double-stranded RNA to name but a few. TLRs also play an important role in the immune response initiated by the DAMPs (damage-associated molecular patterns). The major DAMPs are HMGB1 (high mobility group box protein-1), S100A8/S100A9, heat-shock proteins, uric acid, and DNA.

TLRs are horseshoe-shaped structures that incorporate leucine rich repeats on their extracellular portion. The intracellular segment consists of a TIR domain which transduces activation signals through the adaptor protein MyD88 upon antigen binding. TLRs are initially expressed as monomers, but undergo dimerization upon TLR-ligand binding. The resulting dimer complex is then described as M-shaped (12).

Even though their structural features are highly conserved, TLRs exist in several variations numbered from 1 to 13 depending on the antigens they can bind and the signal they transduce inside cells. The last three types, however, are not found in humans. TLR1, 2, 4, 5, 6 and 10 are expressed at the cell surface while TLR3, 7, 8, and 9 have been shown to localize on intracellular vesicles.



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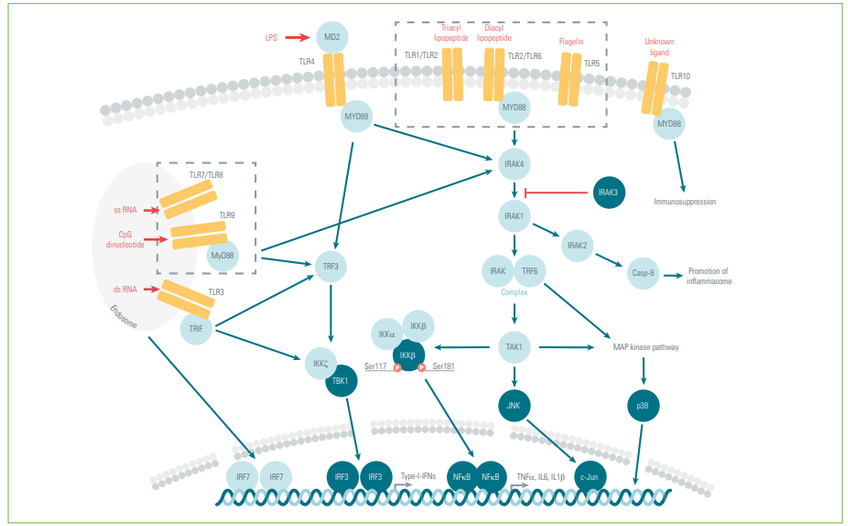
TLR signaling

Activation of TLRs through ligand binding triggers a signaling cascade involving a variety of intracellular signaling adaptors, including MyD88, IRAKs, and TRAF6. TLR signaling leads to the activation of the MAP kinase, IKKs, TBK1, and IRF signaling pathways, which promotes transcription factors including NF-κB, IRF3, IRF7, and Jun. These mediate inflammation through the production of inflammatory cytokines, type I IFN, chemokines, and antimicrobial peptides. TLR signaling in innate immune cells, particularly dendritic cells, initiates their activation and the subsequent induction of adaptive immune responses.

TLR10 is an exception among this receptor family, as it does not promote transcription factors that initiate inflammation but rather represses them. Its effect is therefore immunosuppressive and mediated via ligands that remain unknown to date (13) (14).



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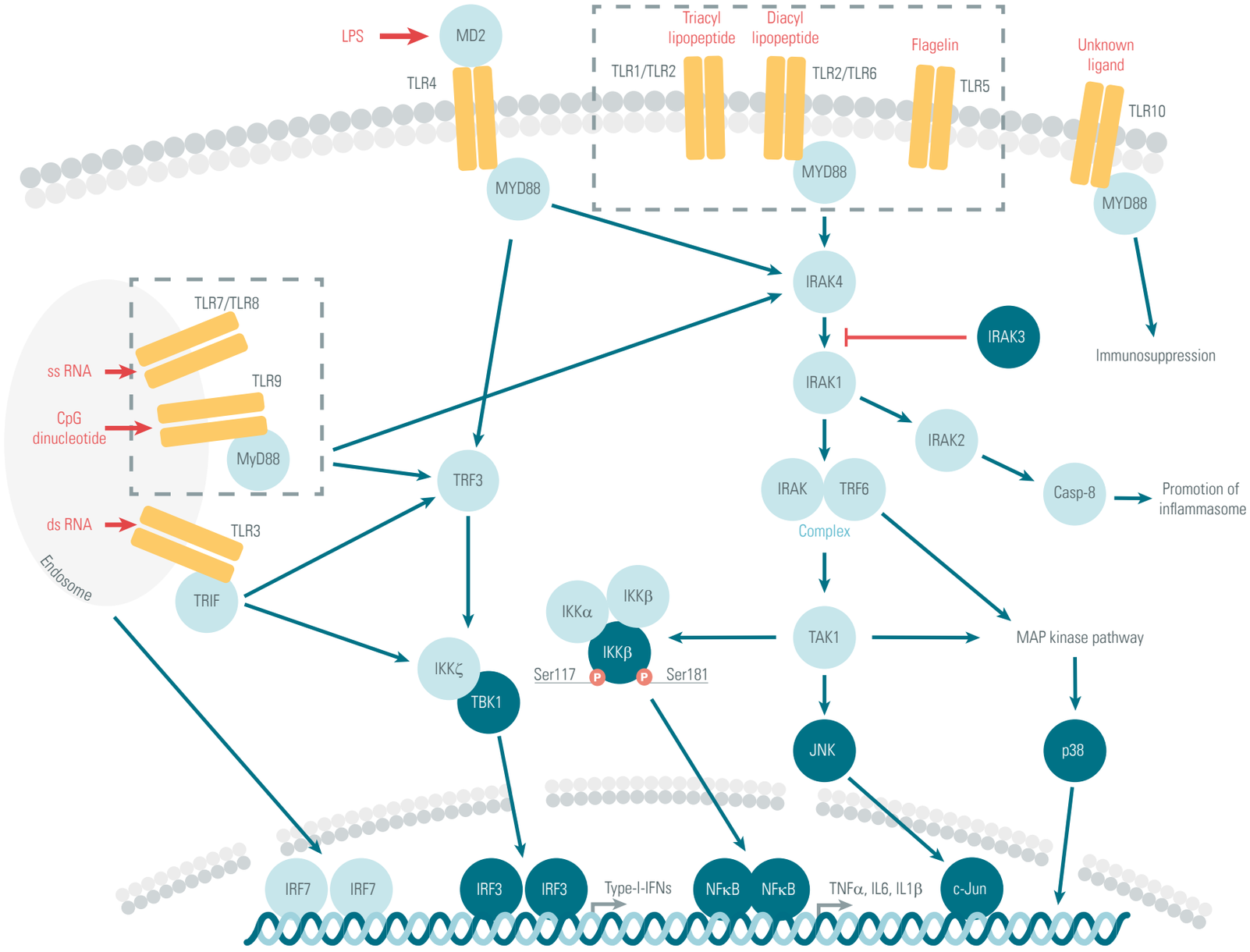

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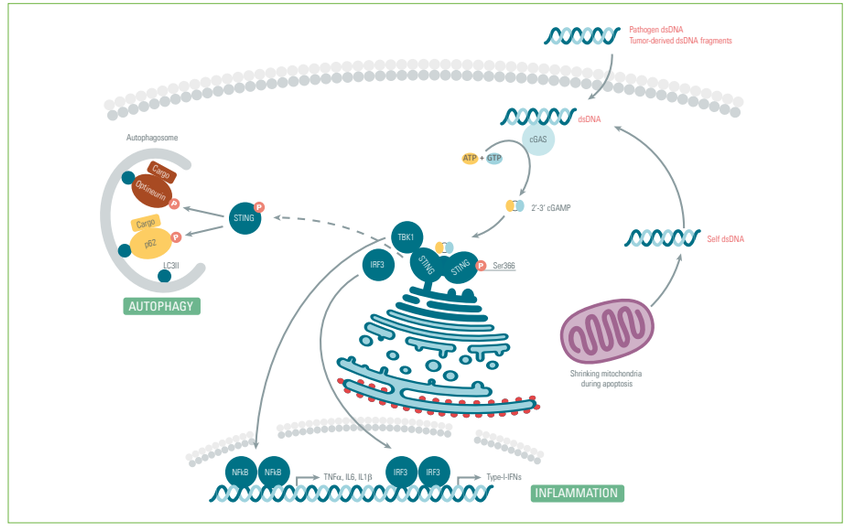
cGAS-STING AXIS

STING, for STimulator of INterferon Genes, is a cytoplasmic homodimeric protein localized in the endoplasmic reticulum and which plays an essential role in innate immunity. Upon pathogen infection or mitochrondrial shrinking during apoptosis, floating dsDNAs bind and activate a cytoplasmic DNA sensor, the cyclic GMP-AMP synthase (cGAS). Activated cGAS leads to the production of 2'-3'cGAMP, a cyclic dinucleotide, which then binds to STING proteins. In turn, phosphorylated STING interacts with and is phosphorylated by TANK-binding-kinase-I (TBK1), leading to the recruitment and activation of interferon regulatory factor (IRF3) dimer. Nuclear translocation of the IRF3 dimer leads to the transcription of genes encoding type 1 IFNs (IFN-α,IFN-β). In addition, the STING pathway controls NF-κB dependent inflammatory cytokine expression through TBK1 signaling. As a negative feedback loop, the DNA-stimulated cGAS-STING-TBK1 pathway also triggers STING protein degradation through p62 SQSTM1 associated autophagy to switch off IFNs production.

In immuno-oncology, tumor derived DNA fragments have been shown to drive host anti-tumor immune response through the STING pathway, and activating this pathway has shown promising anti-tumor effects in pre-clinical models. It thus represents a therapeutic strategy to treat human cancer. It has also been deeply investigated in the context of autoimmune inflammation, as diseases such as lupus and psoriasis are suspected to arise from nucleic acid self-antigens.



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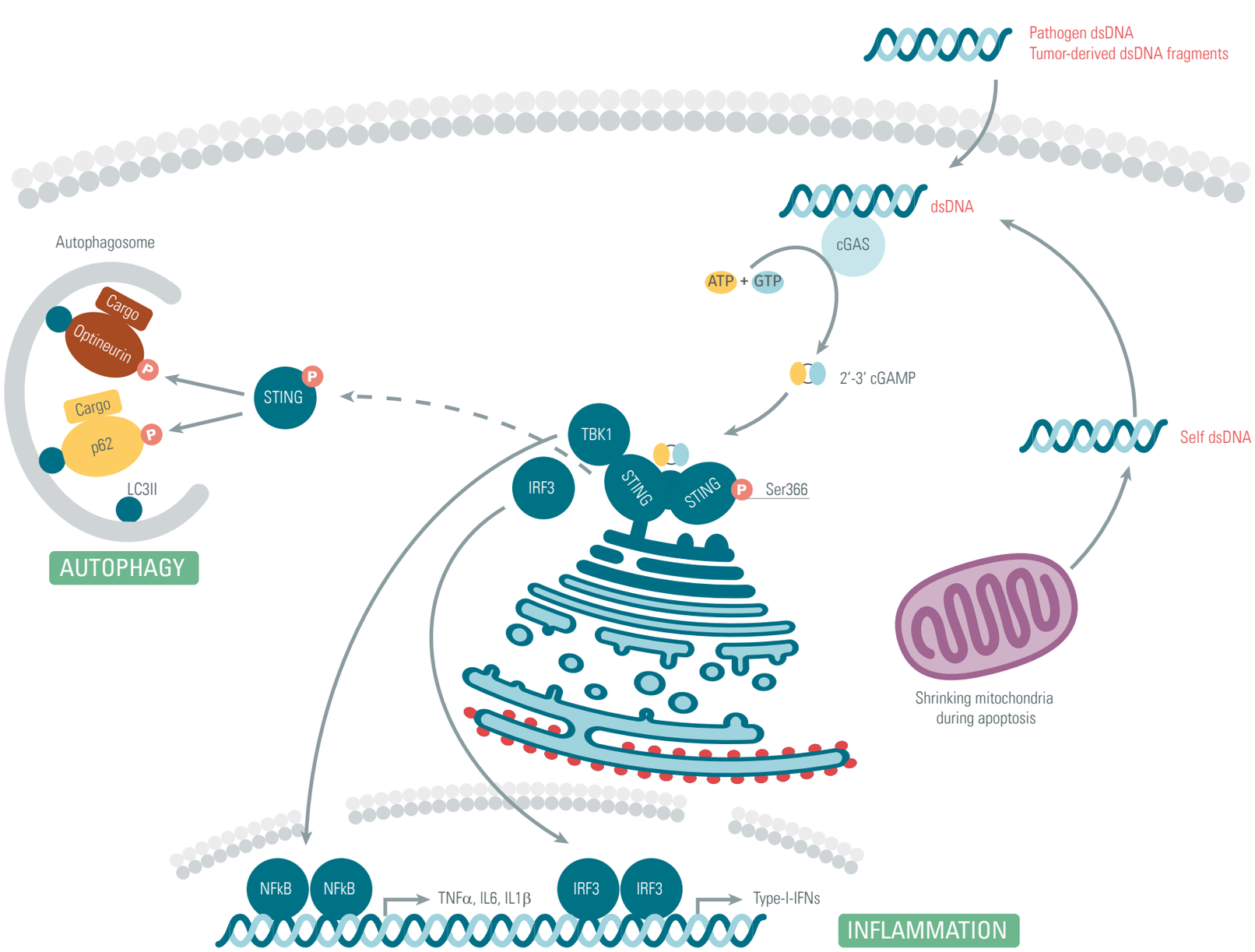

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INFLAMMASOMES

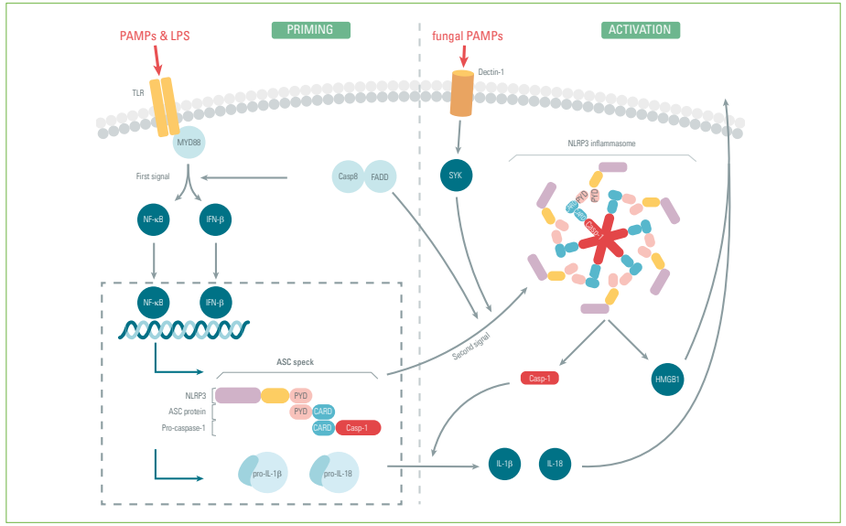
Inflammasomes are a family of cytosolic multiprotein complexes that enable the rapid activation of cytokines IL-1b and IL-18 from their inactive precursors, pro-IL1b and pro-IL18 respectively, and promote the extracellular secretion of the pro-inflammatory alarmin HMGB1. They are part of the innate response and are activated via different stimuli that involve pattern recognition receptors (PRRs) of the innate immunity.

These complexes are usually described as an assembly of identical ASC specks made up of an upstream sensor protein (NLRP 1, 2, 3, 6,or 7, and NLRC4 from the NLR receptor family or AIM2) linked to a downstream effector pro-caspase-1 by an adaptor protein ASC (15). This association is made possible by the matching PYD and CARTD domains of ASC proteins and both partners respectively. Inflammasomes are named after their sensor proteins, hence inflammasome NLRP3, inflammasome AIM2, and others.

Inflammasomes become active following the induction of two signals. The first is a priming signal that promotes the assembly of inflammasomes. TLRs binding their respective PAMPs send transcription factors including NF-kB to the nucleus to stimulate the expression of a variety of proteins, among which are sensor proteins and pro-caspase-1 that assemble in ASC specks, but also pro-IL1b and pro-IL18. This is a way for cells to "sensitize" themselves following the first contact with a pathogen, and to prepare for the probable introduction of foreign material into their cytosol.

The second signal activates. This usually results from sensor proteins binding their canonic ligand (cytosolic DNA, RNA, etc.), which leads ASC specks to assemble into the larger wheel-shaped inflammasome complex and triggers the cleavage of pro-caspase-1 into caspase-1. Active caspases then proceed to cleave stored pro-IL1b and pro-IL18 into their active forms, promoting inflammation both within the cell and around. It has also been shown that this inflammasome assembly and caspase-1 activation regulate and promote the secretion of HMGB1. In the case of NLRP3 inflammasome in anti-fungal response, this activating signal can also come from Syk kinase rendered active by fungal PRRs, such as dectin-1 (16) (17) (18) (19) (20).

Interestingly, caspase-8 and FADD have been observed to associate in a complex that can singlehandedly initiate both signals by promoting the priming of inflammasomes through NF-kB and triggering their cleaving activity (21).



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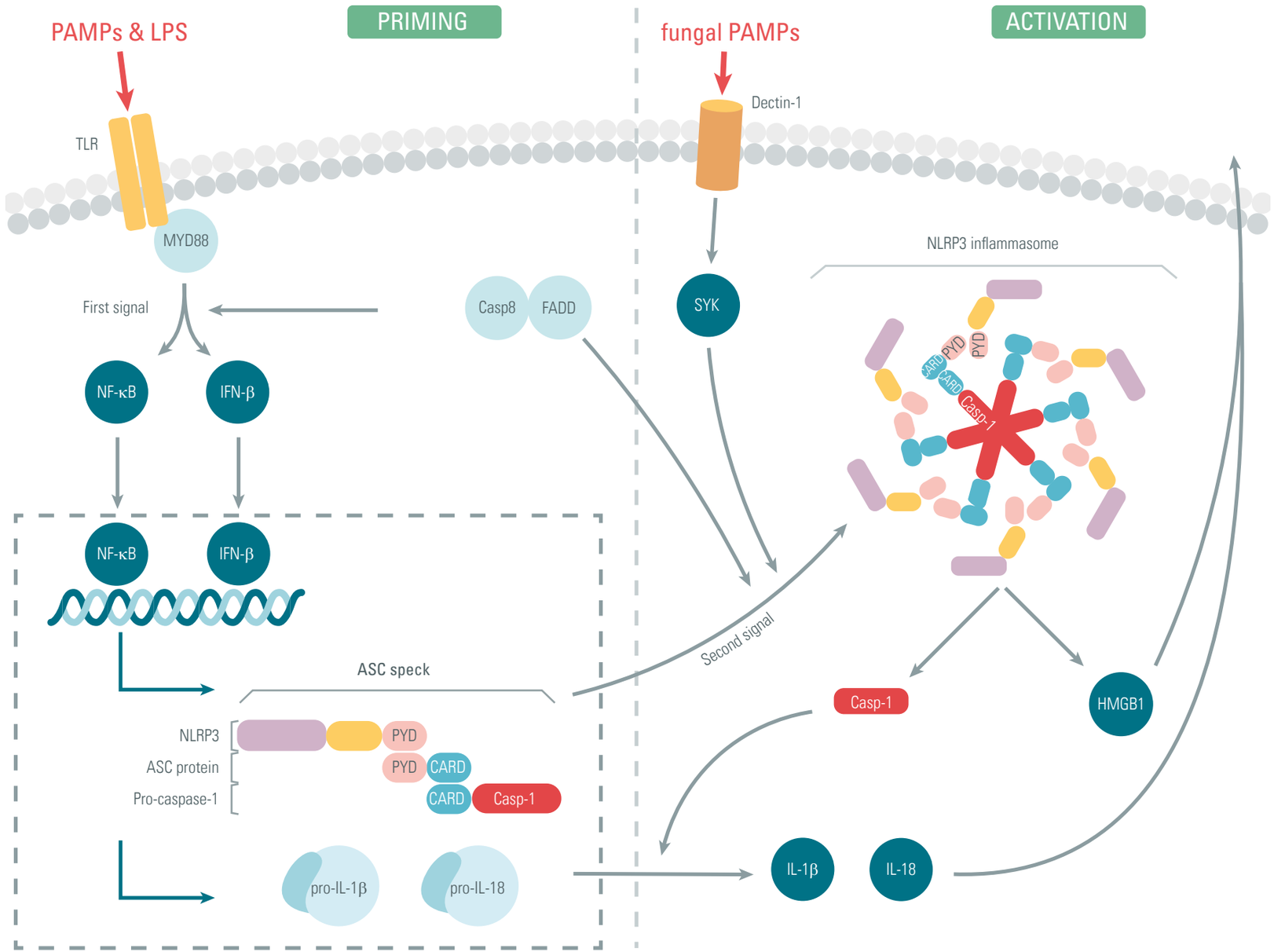

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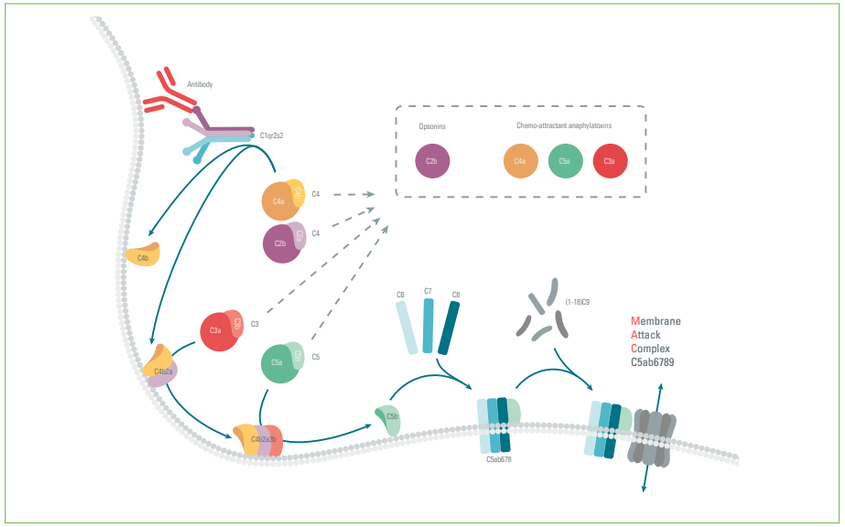
THE COMPLEMENT SYSTEM

The complement system consists of an assortment of small liver-borne proteins circulating as inactive precursors in the blood. It is an actor of the innate immune response, and its activation results in three immune functions:

1. Promotion of phagocytosis via the opsonization of pathogens that drive phagocytes' complement receptors to them (CD35, CD21 or CD11);
2. Direct lysis of gram-negative bacteria and viruses, through the assembly of membrane attack complexes (MAC);
3. Contribution to and sustainability of the inflammation with anaphylatoxins (chemo-attractants and inflammatory peptides (C3a, C4a, C5a).



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ACTIVATION

The complement system has three potential activation triggers. The classical pathway relies on the opsonization of the target with antibodies, and is therefore a crosstalk between adaptive and innate immunity. In such events, the complement 1st factor C1qr2s2 is recruited at the Fc segment of antibodies and initiates a succession of cleaving and recruiting steps with the complement's other factors from C2 to C8. This cascade leads to the polymerization of 1 to 18 C9 factors in a pore-like structure through the target's membrane, which acts like an unregulated transmembrane channel leading to a fatal osmotic stress.

The alternative pathway starts with the spontaneous binding of the complement's factor C3b (an opsonin) to the target, which initiates the classical pathway at this level.

Finally, the lectin pathway relies on the opsonization of the target with Mannose-Binding Lectins (MBL), which assemble in multimers that initiate C4 and C2 cleavage in a similar way to the 1st factor C1qr2s2, and which starts the classical pathway.

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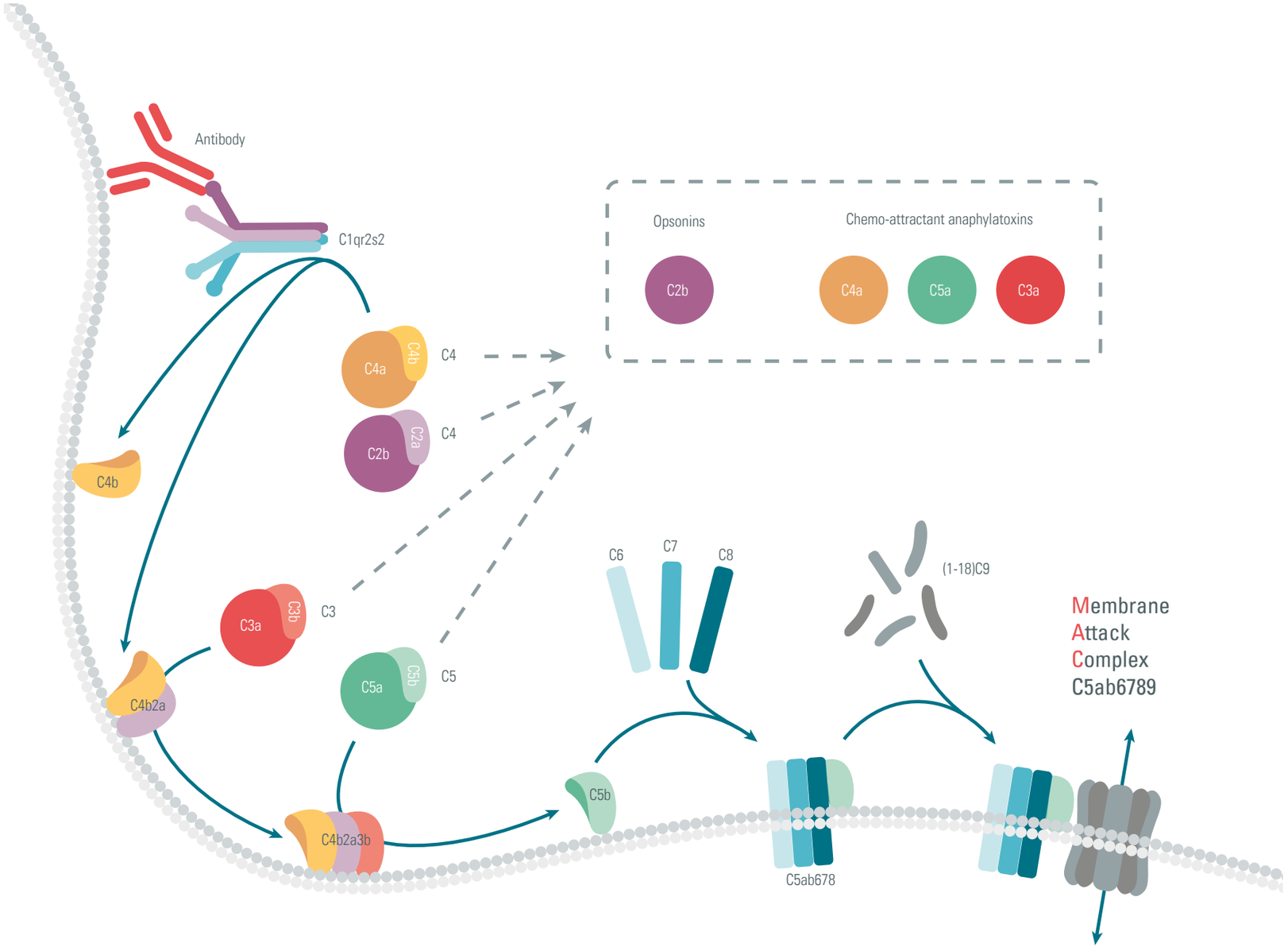

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INNATE IMMUNITY

Innate Immune Cell Types

MACROPHAGES

Macrophages are professional phagocytic immune cells that arise from bone-marrow precursors differentiating into parent monocytes in the peripheral blood. They infiltrate tissues where they become macrophages, and account for 2 to 12% of leukocytes (29). They are plastic cells that respond to their micro-environment and fulfill homeostasis-maintenance missions that include tissue modeling, recycling, and clearance of dead cells and the removal of pathogens such as necrotic debris, bacteria, toxins, etc. Because of their tissue-resident nature, they are usually the first immune cells to confront an intruder, and are responsible for initiating the recruitment of other specialized cells when the threat cannot be easily dealt with.

Macrophages are divided into main categories which exhibit opposed and complementary phenotypes. Classically activated macrophages (CAM) or M1 are induced by a combination of TLR signaling and IFN-γ. They are potent antigen-presenting cells with strong pro-inflammatory cytokine expression (IL-6 & TNF-α), making them key promoters of T-cell expansion and further enhancing their own phenotype. They also exhibit high levels of lysis compounds and regulator nitric oxide that empower them against pathogens. They are the pathogen-clearing pro-inflammatory population of macrophages.

Alternatively activated macrophages (AAM) or M2 are induced by IL-4 or IL-13, and are especially involved in anti-parasite and anti-fungus responses. The fatty acid receptor CD36 has also been identified as a promoter of this phenotype over M1. They express high levels of arginase (enzyme required for DNA synthesis), extracellular matrix proteins, and the immunosuppressive cytokine IL-10. The M2 designation encompasses a third subtype worth mentioning: regulatory macrophages, induced by IL-10 and expressing higher levels of that cytokine than conventional M2 (22) (23).

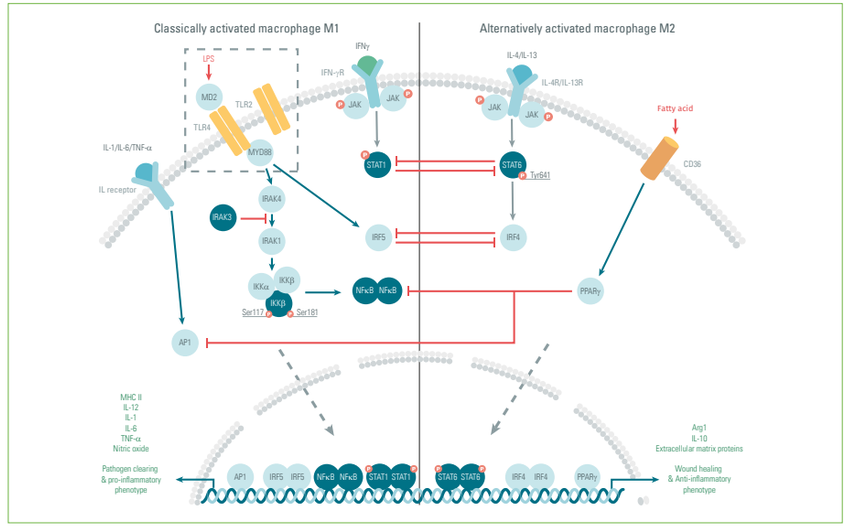
It is important to note that due to their highly plastic nature, macrophage categories of phenotypes are not homogeneous and clearly defined, but rather exist in a spectrum of populations which are not restricted to the characteristics of their most prominent phenotype. Additionally, these main categories are divided into multiple subsets of tissue-specific macrophages whose phenotypes are dependent on the tissues where they reside (microglia in central nervous system, Kupffer cells in liver, osteoclasts in bones, alveolar macrophages in lung, etc) (24) (23).

INNATE IMMUNITY

Innate Immune Cell Types

Macrophage polarization

Macrophage polarization is strongly influenced by environmental factors and mediators which promote the expression of one phenotype while inhibiting the pathway leading to the other. This negative feedback regulation comes from the main transcription factors of both sides, and ensures the expression of a single strongly polarized phenotype. Therefore, a pro-inflammatory context featuring pathogens and IFN-γ promotes the anti-pathogen and pro-inflammatory M1 macrophages through transcription factors STAT1, IRF5, NF-κB, and AP1, which also inhibit the wound-healing and anti-inflammatory M2 macrophages' transcription factors STAT6, IRF4, PPARγ, and vice versa (25).



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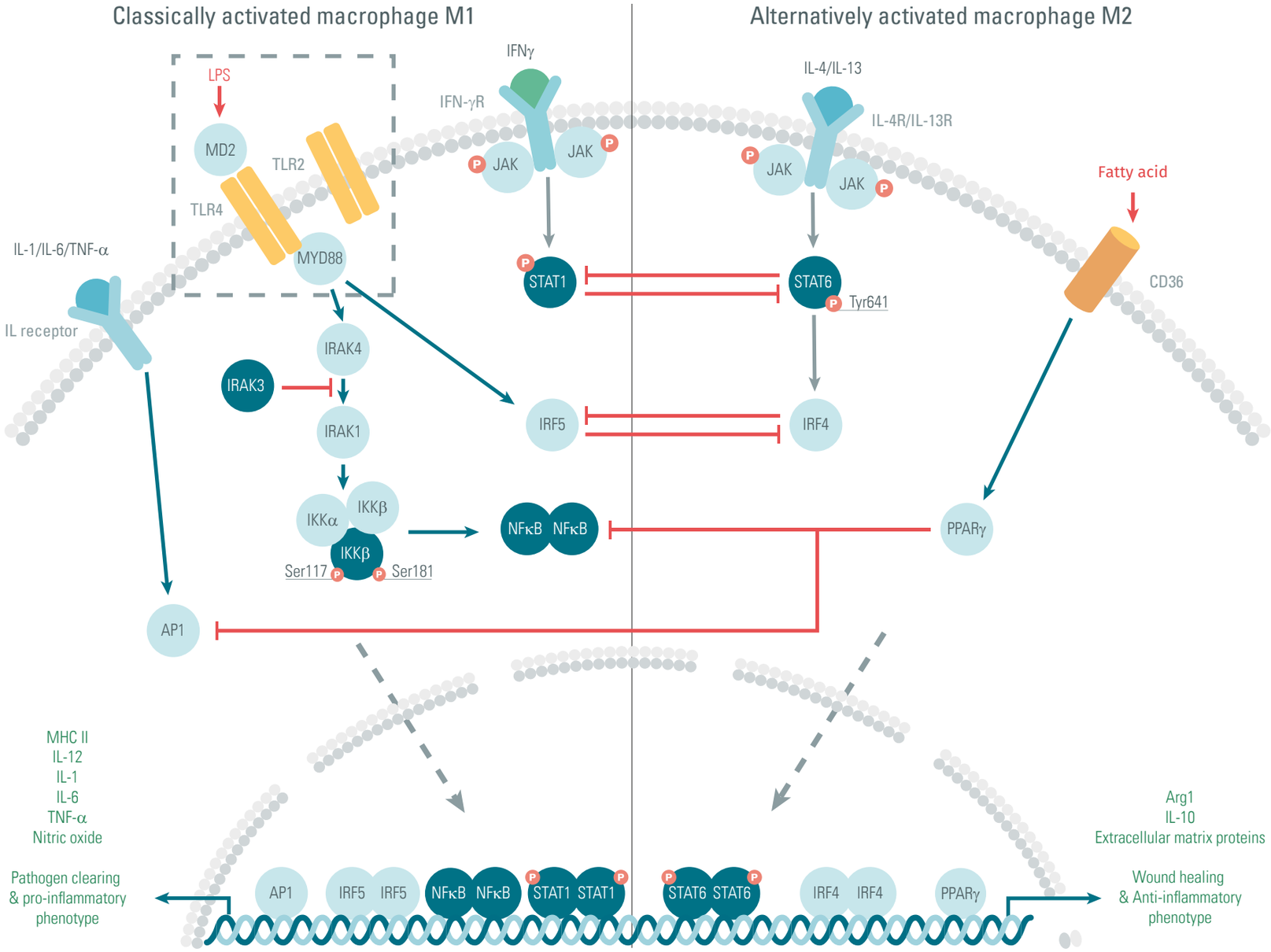

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Innate Immune Cell Types

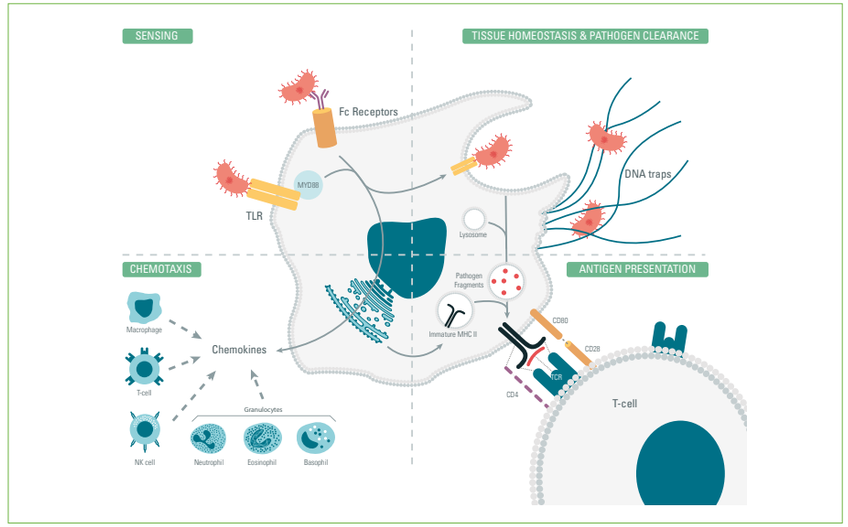
Macrophage functions

Macrophages express an assortment of intracellular and surface pattern recognition receptors (PRRs) which enable them to patrol their environment and sense foreign pathogens and endogenous markers of cell death or degeneration (apoptotic & necrotic markers, modified protein, cellular debris). Their most prominent receptors are TLRs (extra-and intracellular 2 & 4 and 3 & 9, respectively). While most of these receptors promote inflammation through the expression of the pro-inflammatory cytokines IL-6, IL-12, and TNF-α, two specific PRRs (dectin-1 and MRC1) binding β-glucan, mannose, and fucose polysaccharides inhibit the expression of these cytokines, and instead promote the production of the immunosuppressive IL-10. Additionally, macrophages also carry both γ and ε Fc receptors (CD16, CD32, CD64, and CD23) that drive them to antibody-opsonized pathogens and immune complexes. Receptor binding usually leads to phagocytosis of the bound target (pathogen or damaged cells) and to stimulation of the pro-inflammatory functions of macrophages. However, apoptotic or necrotic debris can result in the immunosuppressive and regulatory secretion of IL-10 and TGF-β, to prevent inflammation of the surrounding tissues.

Pathogen sensing leads macrophages to attract, via chemotaxis, the immune cell populations necessary for the management and elimination of the threat. Secreted chemokines and attracted cells are different between M1 and M2, and include Th1, neutrophils, NK cells & other macrophages, and Th2, NK cells, eosinophils, and basophils respectively.

Macrophages are also responsible for preserving the healthy equilibrium state of the tissues they reside in. This includes clearing pathogens that threaten them, but also recycling materials that are either redundant or obsolete in processes such as development, growth, or injury healing. Upon identifying a target, macrophages engulf, phagocyte, and digest it with their own production of reactive oxygen species (ROS). These are regulated by nitric oxide, which reacts with them into toxic nitrogen species suitable for the lysis of pathogens. The arginase production of M2 also promotes the assembly of DNA-made extracellular traps similar to those of neutrophils, which immobilize pathogens and promote their removal (26). As they perform their clearance mission, macrophages promote tissue repair via the secretion of matrix proteins and growth factors.

Finally, macrophages are antigen-presenting cells that promote the adaptive immune response by presenting antigens on their MHC II to stimulate effector cells. However unlike dendritic cells, macrophages are usually unable to prime T-cells, and only stimulate previously activated ones (22) (27) (28).



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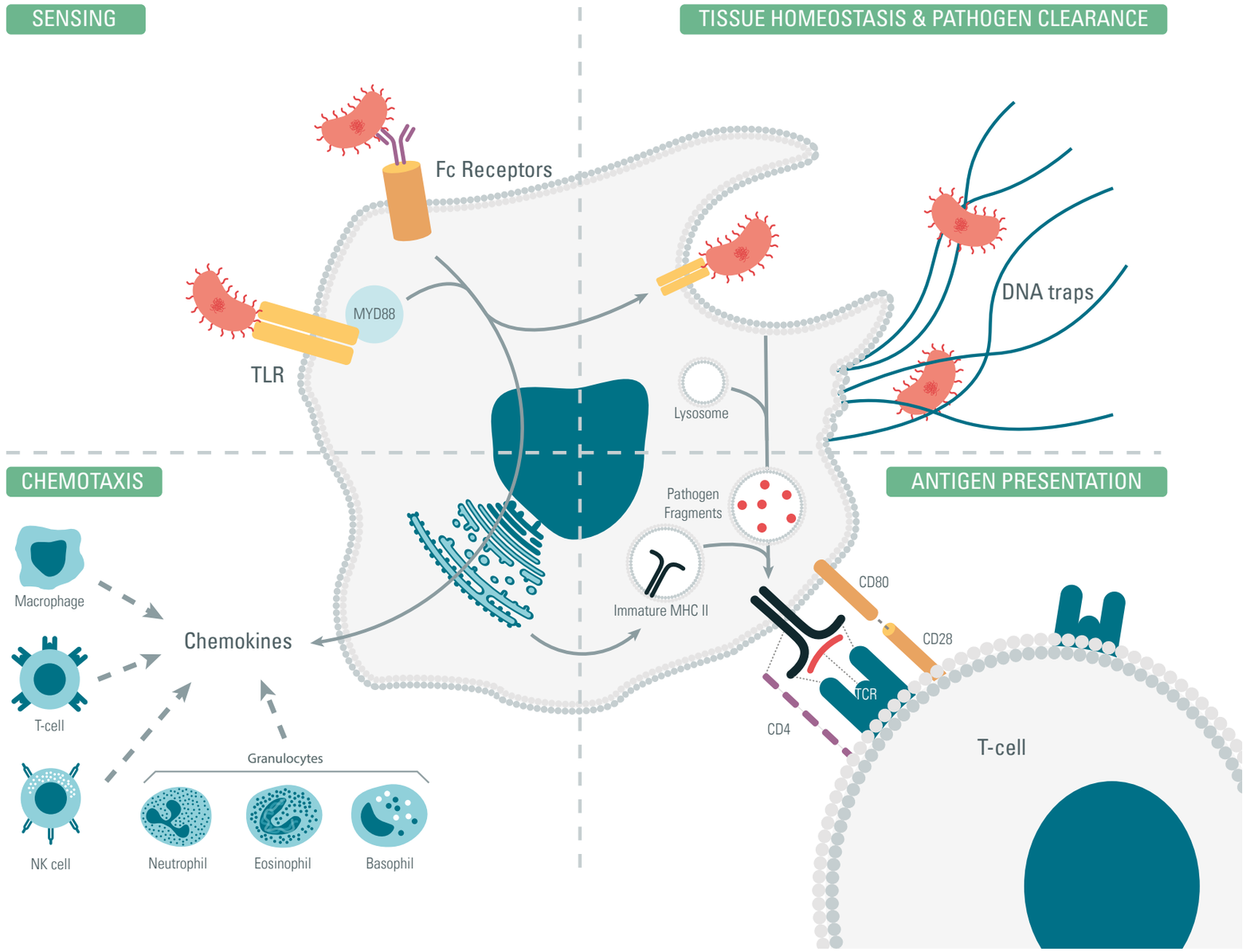

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Innate Immune Cell Types

GRANULOCYTES

Granulocytes are myeloid cells of the innate arm of immunity. They evolve from bone-marrow stem cells and mature prior to entering the blood circulation, which transports them to tissues. They are the most prevalent of leukocytes (35-80%) (29) and are characterized by their poly-lobed nucleus and the cytosolic granules they release upon stimulation. Granulocytes are divided in subsets that have different characteristics and abilities, ranging from pathogen removal, cytotoxicity, and allergy mediation, to immunomodulation. These subsets are called neutrophils, basophils, and eosinophils (acidophils), and are named for their ability to retain acidic eosin and basic hematoxylin stains (30). Regardless of their special features, all three types are involved in the mediation of allergies and inflammation, and are phagocytic cells capable of antigen-presentation.

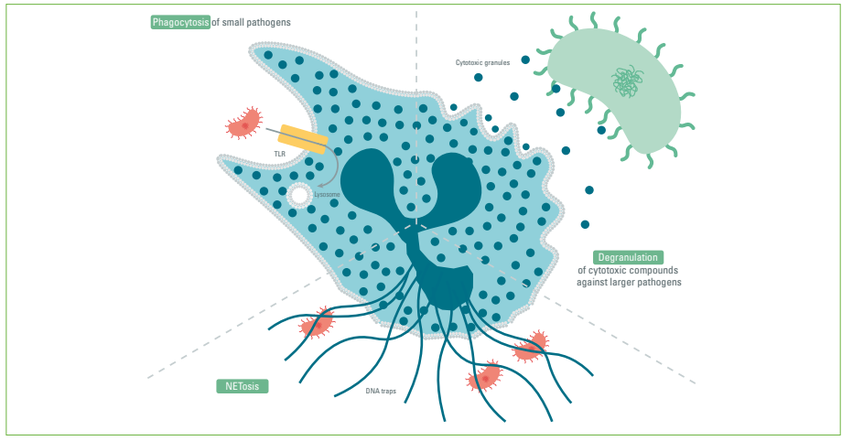
Neutrophils

Neutrophils circulate in blood where they are the most common leukocytes, accounting for 30-80% (29). They are short-lived cells that survive only a few days in blood and tissues, hence their very high renewal rate ranging from 10^{11}/day in a normal state to 10^{12}/day in an inflammatory context (31) (32).

They are phagocytic cells and clear small pathogens via intracellular lysosomal degradation. However, they can effectively attack all kinds of large pathogens with the proteolytic enzymes, cytotoxins, antimicrobial peptides, and other molecules contained in their granules (33). Neutrophils are typically the first immune cells recruited at the infection site when the local macrophages become overwhelmed and signal for help with their chemotactic secretions. In such an immune context their lifetime is prolonged, leading to their accumulation at the infection site and the rise in the inflammatory and chemotactic signals they emit collectively. This in turn promotes the activity of local macrophages and mast cells to handle the intruder and recruit other immune cells to constitute a more developed and intense immune response.

A specific anti-pathogen strategy which neutrophils use and share with eosinophils and macrophages is the assembly of DNA structures into extracellular nets that act as traps to capture, neutralize, and promote the removal of pathogens (Neutrophil Extracellular Traps) (34) (35).

Neutrophils are highly plastic and have been observed to adopt various and sometimes opposite and counterintuitive phenotypes. This is especially significant in the field of oncology, where two subsets of tumor-associated neutrophils N1 and N2, which respectively exhibit anti- and pro-tumor activities, have been shown to polarize differently depending on the acute or chronic inflammatory state of their micro-environment. This polarization and its possible reversibility are currently active areas of research in immuno-oncology (36) (37).



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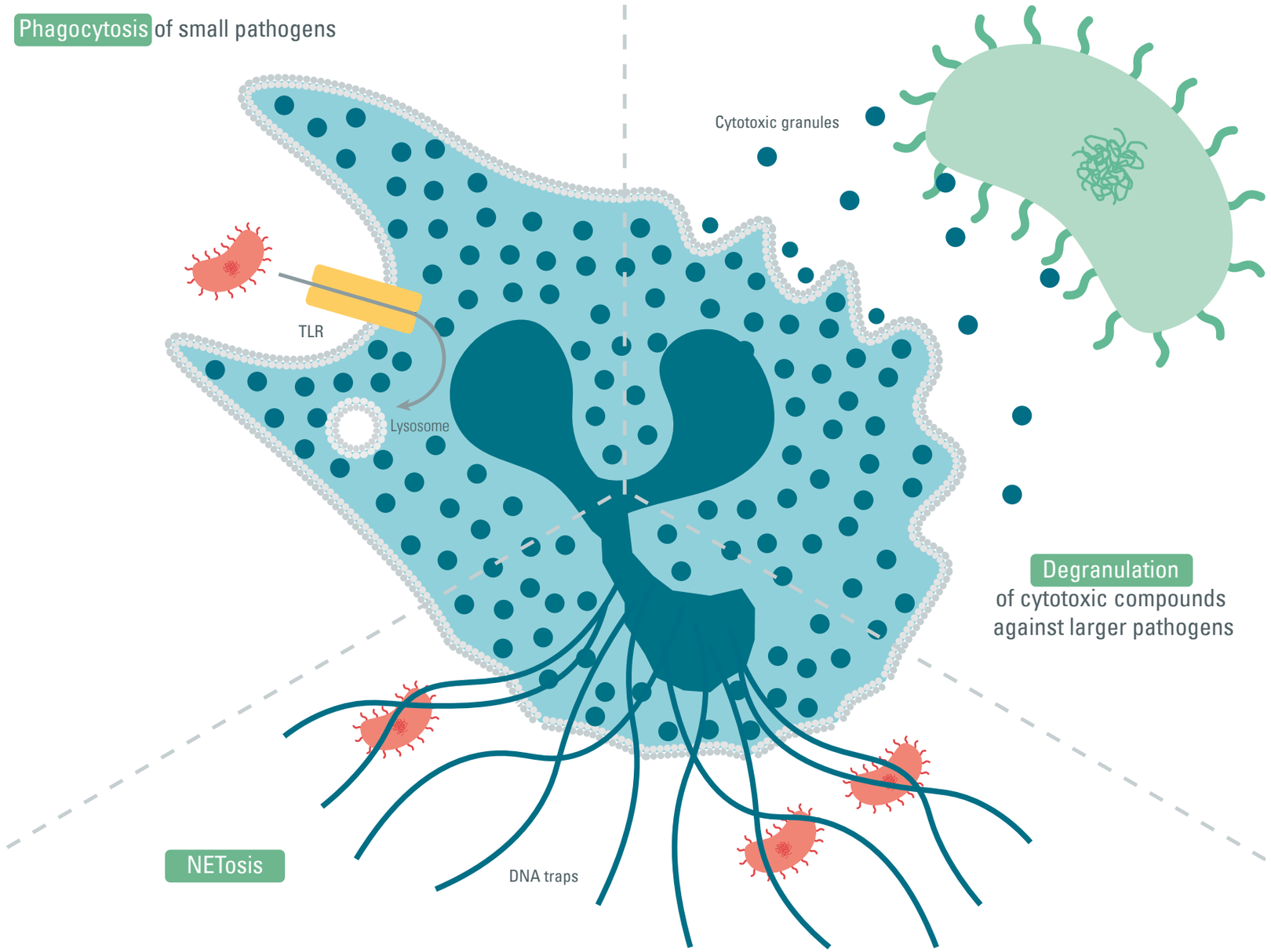

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INNATE IMMUNITY

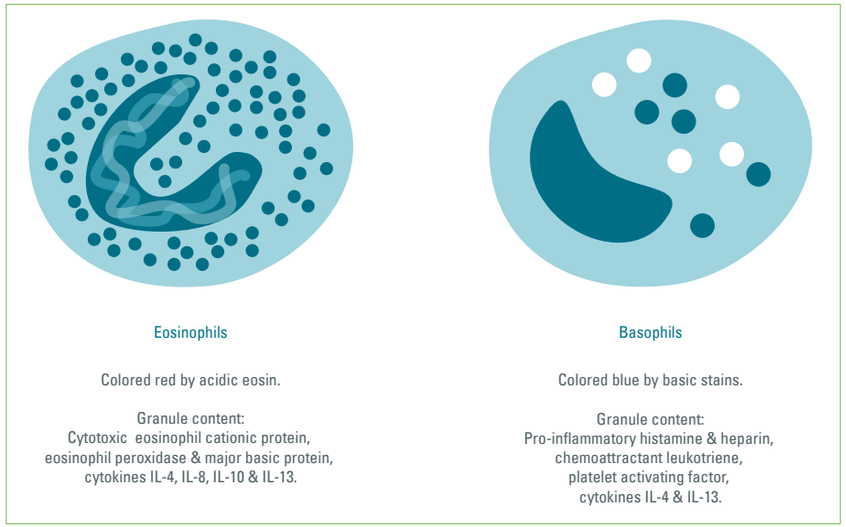
Innate Immune Cell Types

Eosinophils

Eosinophils account for 0-7% of leucocytes (29), and mostly reside in epithelia in normal conditions. Similarly to neutrophils, eosinophil granule content includes cytotoxic compounds (eosinophil cationic protein (ECP), eosinophil peroxidase (EPX), and major basic protein (MBP)) that enable the extracellular destruction of most kinds of pathogens. However, in these cells the granules also contain cytokines such as IL-4, IL-8, IL-10, and IL-13 which enable them to modulate both innate and adaptive immunity as they perform their pathogen clearance missions. As phagocytes, they also fulfill antigen-presenting missions and are known to contribute to T-cell activation and polarization (38) (39) (40) (41).

Basophils

Basophils are the rarest type of granulocytes, accounting for only 0-2% of leucocytes (29), and mostly circulate in peripheral blood, where their lifespan is the shortest among the granulocytes (1 to 2 days in normal state). They are usually recruited in tissue upon inflammation and allergic disorders. Unlike other granulocytes, they do not kill pathogens with their granules' content. However, they are strong modulators of inflammation and immune cells in allergic contexts, because of their inflammatory histamine and anticoagulant heparin content (lesser amounts than in mastocytes), chemoattractant leukotriene (LTs), and platelet activating factors (30) (38). They also retain a phagocytic activity which is especially efficient against IgE immune complexes, which they bind with their FcεRI receptors. Despite their similarity, basophils are not blood-circulating precursors of mast cells (42). When active, basophils express high levels of IL-4 and IL-13 (42), which modulate allergic response, dendritic cell activity, and macrophage polarization toward tissue repair. They have been observed to contribute to Th2 differentiation in certain situations, but are not required for that process (38) (43).



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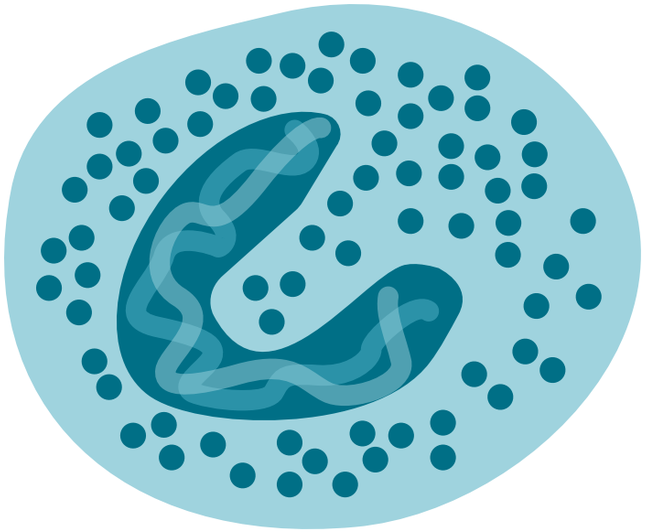
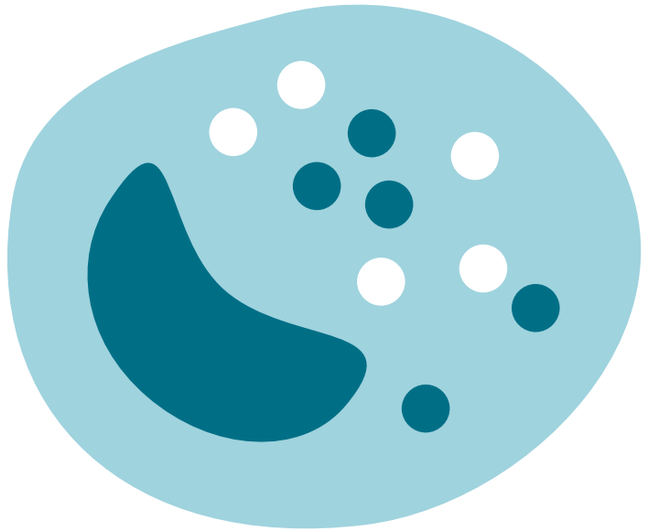


Eosinophils

Colored red by acidic eosin.

Granule content:
Cytotoxic eosinophil cationic protein, eosinophil peroxidase & major basic protein, cytokines IL-4, IL-8, IL-10 & IL-13.



Basophils

Colored blue by basic stains.

Granule content:
Pro-inflammatory histamine & heparin, chemoattractant leukotriene, platelet activating factor, cytokines IL-4 & IL-13.

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INNATE IMMUNITY

Innate Immune Cell Types

MASTOCYTES

Identified as early as 1878 (44), mast cells or mastocytes are a family of bone-marrow borne hematopoietic cells (45) (46) (47) largely known for their role in allergic reaction and anaphylactic shocks. They are mostly responsible for these events through their propensity to quickly release massive amounts of histamine and heparin upon stimulation (48) (49).

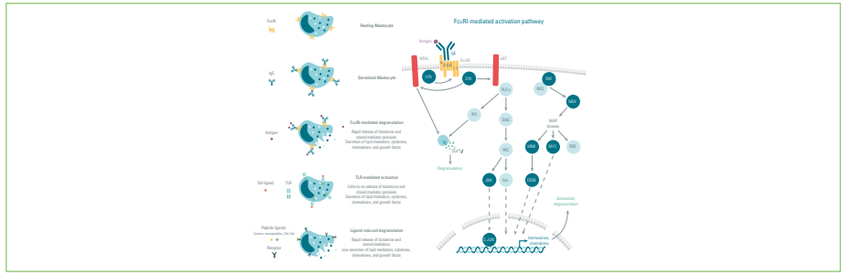
They are a highly plastic immune cell type, widely spread among multiple tissues and interfacing barriers. They mature and express various phenotypes depending on their microenvironment and host tissues (50) (24). Interestingly, they only exist in blood as progenitors (51). Mast cells act as sentinels that have quick reactions to their environment and respond with delicate balances of active compounds. Consequently, any mis-regulation of their behaviors contributes to multiple allergy-related and inflammatory disorders or autoimmunity pathologies and cancers (52) .

Activation and function

Mast cells can activate in three ways. The first and most characteristic is FcεRI-mediated. Resting mast cells express significant amounts of FcεRI, the high-affinity IgE receptor. In presence of IgE, mast cells cover themselves with them and become sensitized (24). Upon IgE binding to an antigen, the cytoplasmic ITAM portions of FcεRI recruit and are phosphorylated by Lyn kinase, then recruit Syk kinase and trigger its autophosphorylation and subsequent activation. Syk kinase phosphorylates transmembrane adaptors LAT and NTAL, which serve as platforms for other signaling partners. LAT enables PLCγ (Phospholipase C gamma) to produce IP-3 and DAG (diacylglycerol), which lead to intracellular calcium influx and PKC activation. LAT is also responsible for initiating the MAP kinase pathway by activating MKKK. NTAL is a promoter of calcium influx through the activation of PI3K (Phosphatidylinositol-4,5-bisphosphate 3-kinase) (53). The FcεRI-mediated activation results in transcription factor activation and transduction of signals to the nucleus, leading to the fast degranulation (massive histamine release) of the cell and the expression of cytokines, anaphylatoxins (C3a & C5a), and lipidic mediators (24).

Other than FcεRI, mast cells express a variety of receptors able to initiate a ligand-induced activation by binding a collection of molecules such as adenosine, C3a, C5a, immune complexes, chemokines, PAMPs, etc. These receptors stimulate the FcεRI-mediated degranulation through different early pathways that converge on the release of the mediators. While such pathways are not well described, it has been suggested that some could involve the activation of the SRC-family kinases (among which Lyn), which are tied to a portion of the FcεRI-mediated pathway.

Finally, as sentinel cells of innate immunity, mast cells express TLRs that enable them to identify large antigen class PAMPs and DAMPs. This TLR-mediated activation is transduced through the regular NF-κB pathway, and differs from the two others by resulting in little to no histamine degranulation. Cytokines, anaphylatoxins, and lipidic mediators are shared with FcεRI- and ligand-induced activations (50) (24).



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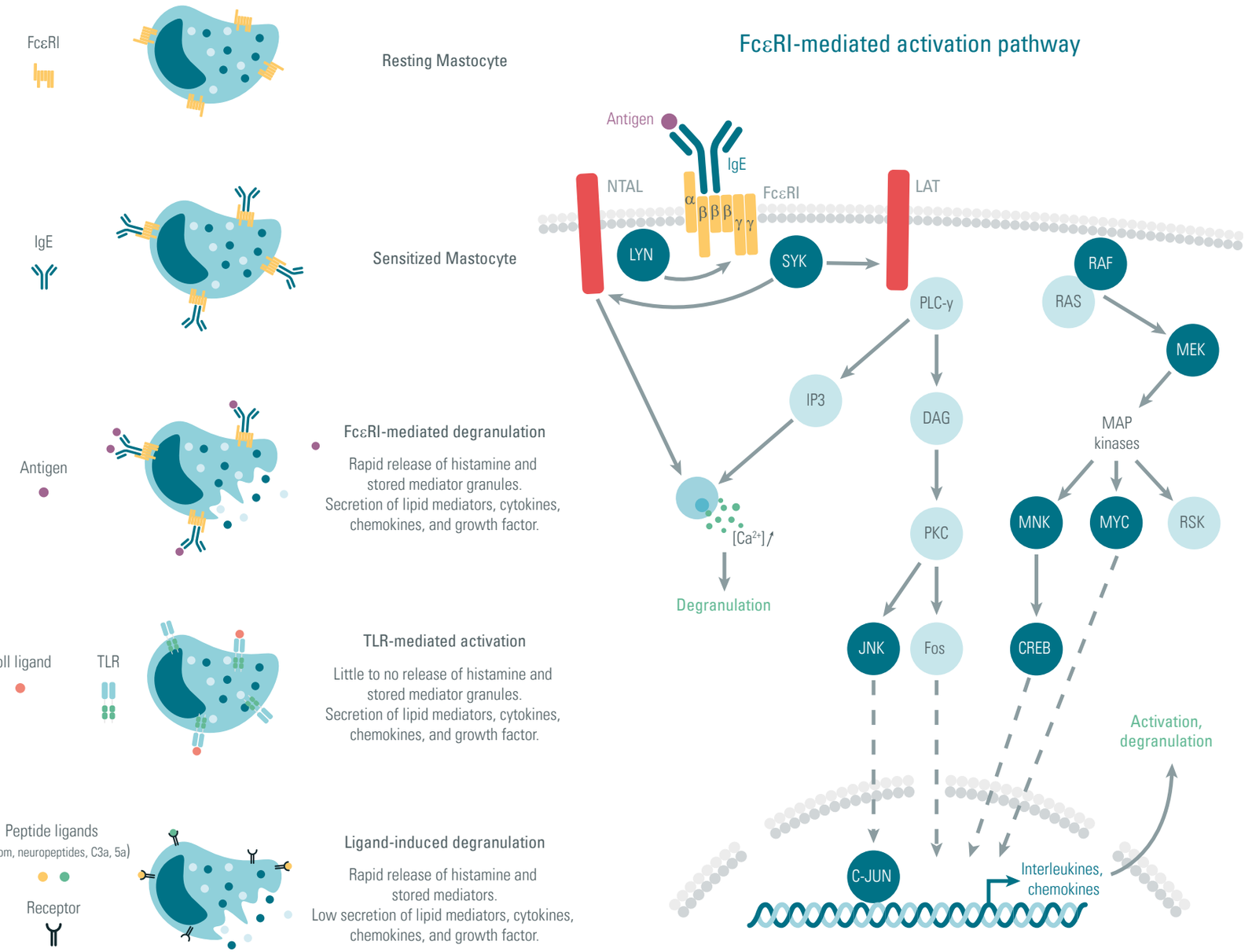

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INNATE IMMUNITY

Innate Immune Cell Types

DENDRITIC CELLS

Dendritic cells (DCs) are bone-marrow-derived cells suspected to come from both myeloid and lymphoid lineages (54), and accounting for 0.3 to 0.9% of leucocytes (29). They are found in blood, lymphoid tissues, and epithelia (skin, liver, mucosa), and act as innate immunity sentinels. They orchestrate the development of the adaptive immune response by capturing antigens and presenting them to other immune cells for priming and activation. They also have secretion abilities in the range of interferons and pro-inflammatory cytokines that promote the recruitment and contribution of other players in the immune response.

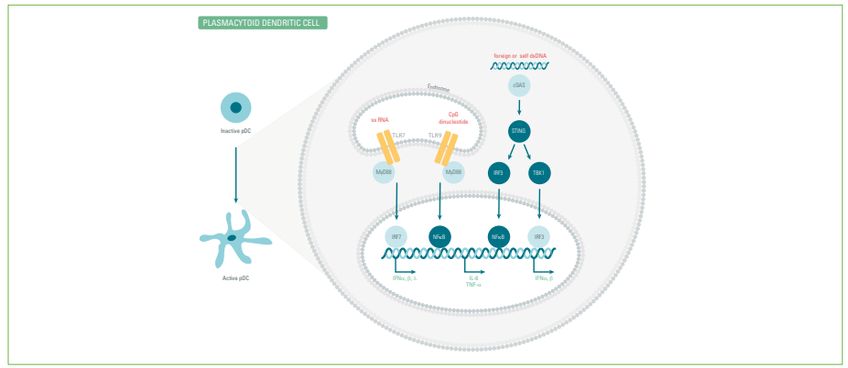
DCs bind antigens in two ways. In an early encounter they rely on innate immunity pattern recognition receptors (PRRs) which target pathogen associated molecular patterns (PAMPs) or damage associated molecular patterns (DAMPs), found in micro-organisms and pathologic cells of self, respectively. In the humoral immunity context, dendritic cells are efficient at clearing antigens via their arsenal of Fc receptors which drive them to antibody-opsonized antigens (55).

Following antigen capture, dendritic cells phagocyte and digest it into fragments presented on their MHC I and MHC II (cross-presentation). They then detach from their tissues, and migrate to lymphoid organs to interact with B and T lymphocytes and prime them to promote an adaptive response.

Ontogenic investigations have split DCs into two categories: plasmacytoid DCs (pDC), and classical/conventional or myeloid DCs (cDC). Distinctions are based on lineage and transcription factors (IRFs), and are considered consistent across all mammalian species (56).

Plasmacytoid dendritic cells (pDC)

pDCs are the most numerous dendritic cells in blood (57) (56). They are largely found in lymph nodes, where they account for roughly 20% of MHC II+ cells (58). They exhibit outstanding secretory abilities of type 1 and 3 interferons (IFN-α, -β, -λ) in response to the detection of intracellular nucleic acids (ss-DNA and CpG-DNA) through their overexpressed toll-like receptors, TLR7 and TLR9 (56) (59). They have also been reported to detect internal nucleic acid via the cGAS-STING axis (60). These sensory abilities enable the identification of viral and bacterial infection, but can also trigger autoimmune disorders that stem from self-DNA being released and present in the cytosol (61) (62). pDC activation following TLR stimulation results in the usual products of TLR pathways. In such situations, IFN production is mainly mediated by the transcription factor IRF7, while pro-inflammatory TNF-α and IL-6 depend on NF-κB signaling. Due to the role of IFN in the initiation of inflammation response and the sensitivity of pDC to nucleic acids, either through their TLRs or the cGAS-STING axis, this cell type could play a triggering role in the pathogenesis of autoimmune diseases such as psoriasis or Lupus (63).



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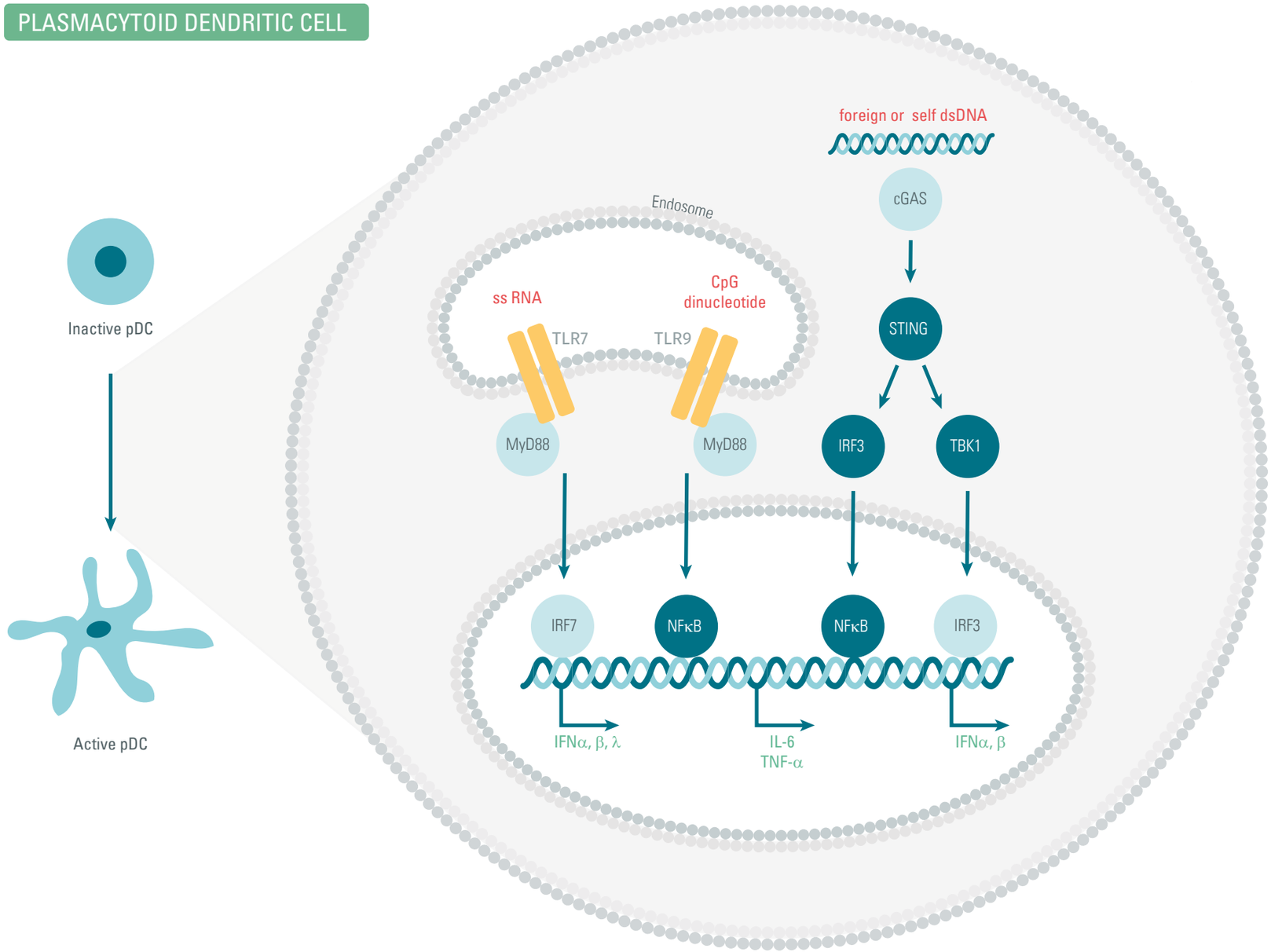
PLASMACYTOID DENDRITIC CELL



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INNATE IMMUNITY

Innate Immune Cell Types

Classical/conventional dendritic cells

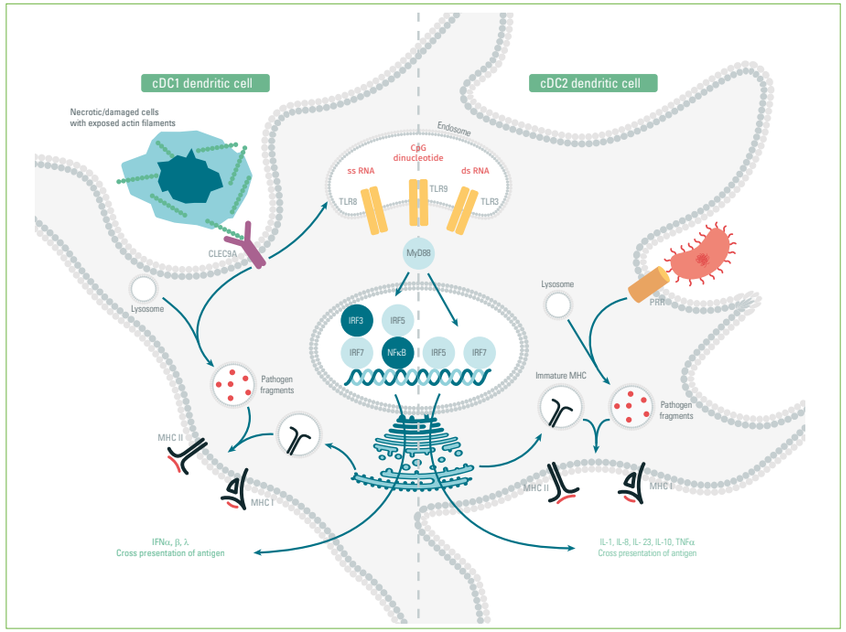
Also called myeloid, these are divided into subsets more focused on antigen-presentation and T-cell priming and activation than their plasmacytoid cousins (56).

Classical/conventional dendritic cells 2 (cDC2), formerly known as CD1c+ DCs, are a the most numerous type of classical DCs and account for 0.6% of PBMCs. They are especially found in tissues, blood, and lymphoid organs such as lymph nodes, tonsils, and spleens, and are interestingly more active in tissues than in blood.

cDC2 carry a collection of lectins, TLRs, and other PRRs that help them carry out their innate immunity roles. This variety of receptors makes them sensitive to lipopolysaccharides, flagellin, poly IC, and other microbial patterns covering a range that includes glycoproteins and glycolipids, and enable the detection of bacteria, mycobacteria, and possibly fungi (56) (64) (65) (66). When stimulated, they express IFNs and numerous cytokines including IL-23, IL-1, TNF-α, IL-8, and IL-10. They are effective antigen-presenting cells that endocyte and digest the pathogens they capture and present them on both their MHCs (cross-presentation). This makes them key players in the priming of T-cells, whether CD8+ T-cells or CD4+ T-cells, which they have been observed in vitro polarizing into helper Th1, Th2, and Th17 (56).

Classical/conventional dendritic cells 1 (cDC1), formerly known as CD141+ dendritic cells, are the rarest subset of DCs and only account for about 0.05% of PBMCs. They are spread across the same tissues as cDC2, including some non-lymphoid tissues such as skin, lungs, intestine, and liver (56) (67).

The cDC1 population is vital in detecting viral infection thanks to their lectin CLEC9A, which recognizes the actin filaments exposed on cells undergoing necrotic cell death. This enables the uptake of material from these damaged cells, and makes cCD1 especially good at identifying infections or disorders leading to such damage (67) (56) (68). Additionally, their TLR3 and TLR9 detect ds-RNA and DNA, and mediate the expression of type 1 and 3 IFN. cDC1 are especially major secretors of IFNγ. Once activated, they contribute to the stimulation of helper T-cells and NK cells with IL-12 expression, and are highly effective antigen-presenting cells capable of cross-presentation on both MHC types, giving them a key role in priming both CD4+ and CD8+ T-cells (56).



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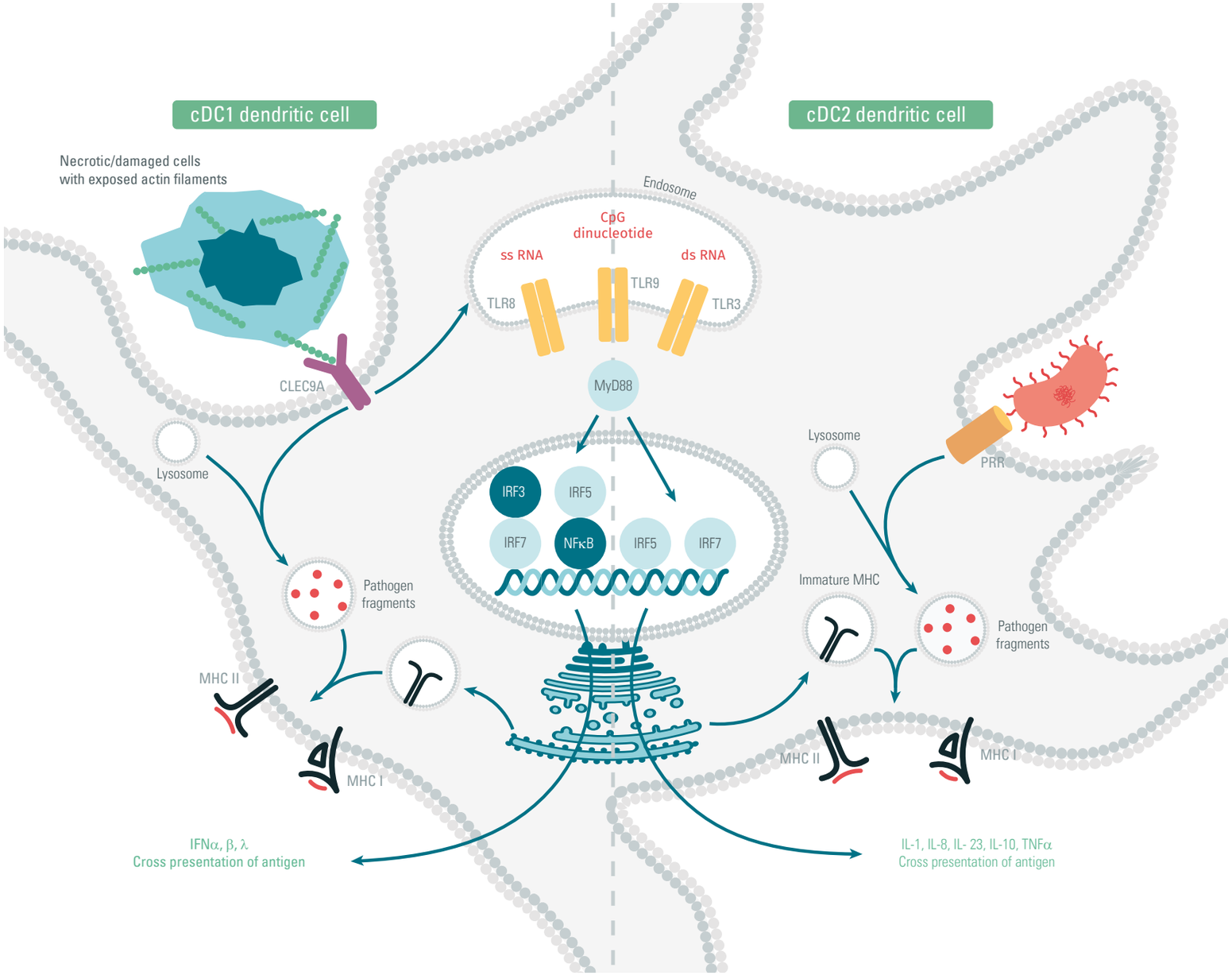

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INNATE IMMUNITY

Innate Immune Cell Types

NATURAL KILLER CELLS (NK)

Natural Killer cells (NK) are a subset of lymphoid cells that evolve from the same progenitor as B and T cells. They are described as large granular lymphocytes originating from the bone marrow (69) and account for 1 to 6% of leucocytes (29). They are known for their ability to target and lyse a wide spectrum of tumorous, infected, or damaged cells, without prior activation and in a non-specific fashion (70). This makes them critical in dealing with degenerated cells which do not express MHC I (such as red blood cells) and therefore cannot be addressed by T-cells.

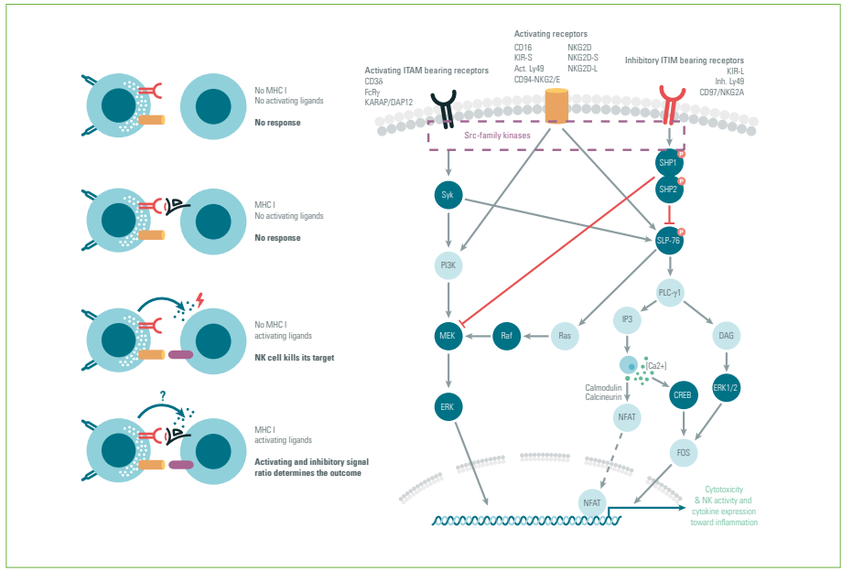
NK cells perform their cytotoxic role via the granzyme/perforin axis, much like CD8+ T-cells. They also act as sentinels ready to detect degenerated cells early on and to stimulate adaptive immunity by expressing inflammatory cytokines (TNF-α and IFN-γ). This rapid pro-inflammatory reaction of NK cells is made possible by their constitutive expression and storage of IFN-γ RNA transcripts, which are readily translated upon cell activation (71).

Characterization

In humans, NK cells are split in 2 subsets depending on their cell-surface density of CD56 (CD56dim & CD56brigt). The two types differ in their properties, and each is more prone to fulfill either a cytotoxic or a cytokine secretion role (72). They do however share the activating receptors NKG2D and natural cytotoxicity receptors (NCRs) NKp30 and NKp46 (73) (74), which are suspected to bind a variety of ligands upregulated in stressed, damaged, and tumorous and transformed cells (75)

$CD56^{dim}$ NK cells are the most abundant subset and account for over 80% of peripheral blood NK cells (76). They are more likely to perform a cytotoxic activity than their CD56bright cousins. To fulfill this role, they synthetize and store perforins and granzymes in granules, and express high levels of Ig-like NK receptors (KIRs, CD158a, CD158b, NKB1) and CD16 which drive them to antibody-bound pathogens in ADCC. However they exhibit weaker cytokine secretion potential than their CD56bright counterpart (77).

$CD5^{6bright}$ NK cells account for 2-14% of peripheral blood NK cells (76), and are mostly found in lymph nodes and tonsils (78). They exhibit a weak cytotoxicity in their normal state but can enhance it following IL-2 or IL-12 stimulation (72) (79). This initially low cytotoxicity results from their lack of perforins (77) and their low to absent expression of CD16 and KIRs (78) (72) (77). They produce abundant cytokines upon activation, including TNF-α, IFN-γ, GM-CSF, IL-10, IL-13, and TNF-β (80)



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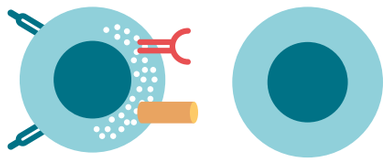
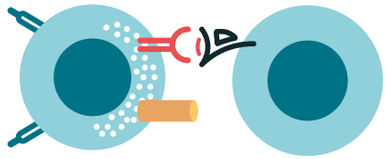


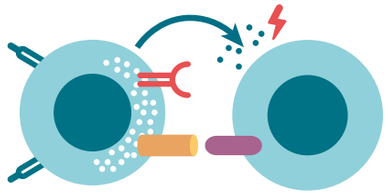
No MHC I
No activating ligands

No response



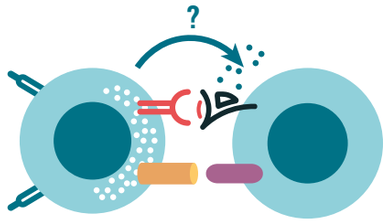
MHC I
No activating ligands

No response



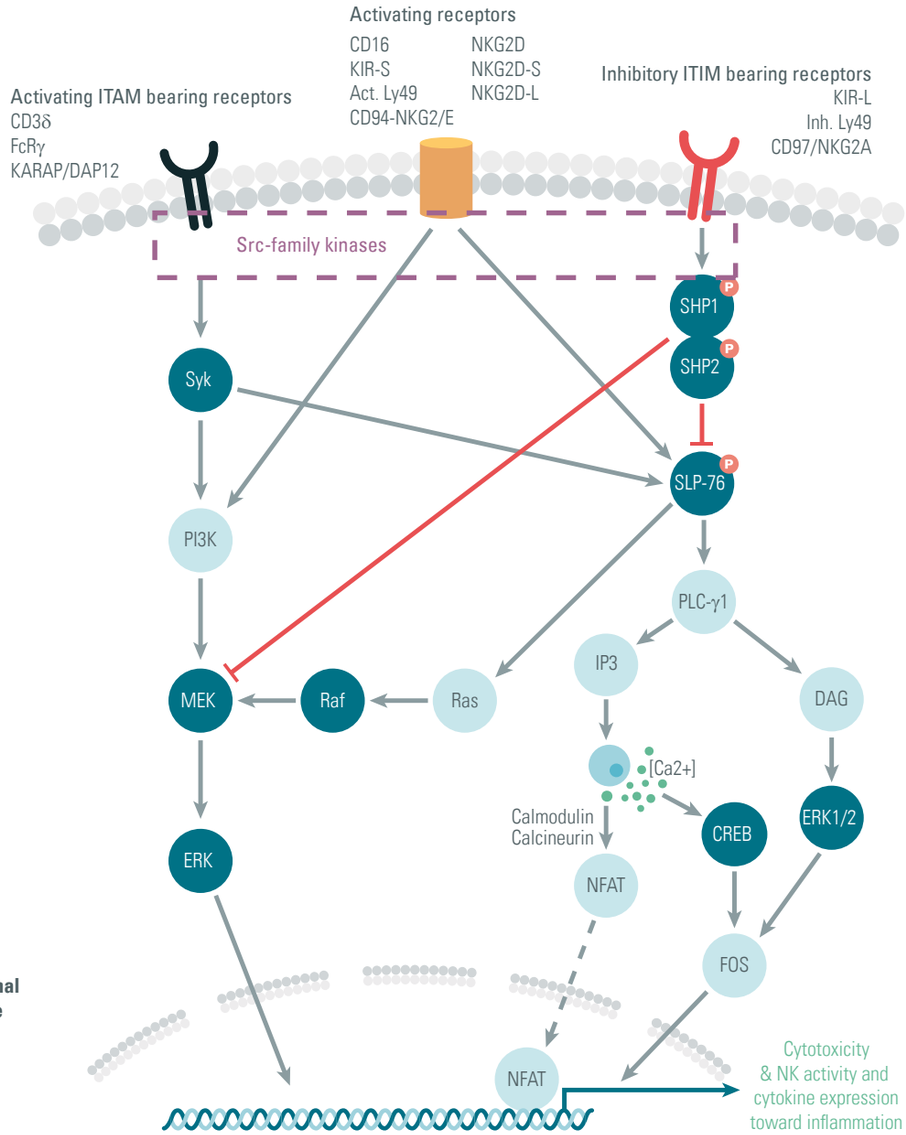
No MHC I
activating ligands

NK cell kills its target



MHC I
activating ligands

Activating and inhibitory signal ratio determines the outcome



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INNATE IMMUNITY

Innate Immune Cell Types

Natural killer cell functions

Decision-making process of the cytotoxic function

The NK cell activation mechanism was initially described with the "missing self" hypothesis, which states that NK cells target cells which fail to express MHC I. It has been proposed that this mechanism greatly contributes to the identification of tumor cells and transformed cells, since these are known to downregulate the expression of MHC I (81) (82). Later the notion of an activating/inhibitory signal ratio was introduced to include evidence of NK activation by MHC I-expressing cells, and NK are now described as expressing a collection of activating and inhibitory receptors that guide their decision-making process by respectively binding molecules expressed on the surface of tumorous or infected cells and MHC I or inhibitory ligands (83).

NK cells constantly move onto the surface of other cells, testing them with their receptors. The decision to kill or spare is based on the ratio of activating and inhibitory signals they receive from their interlocutor. The simultaneous binding of multiple activating receptors can override the signal of inhibitory receptors binding MHC I, and trigger NK cell cytotoxic activity (84). The same override phenomenon has been observed when a potent enough activating receptor is bound (85). The case of self and healthy cells expressing low or no MHC I, such as red blood cells and neural tissues, is not fully understood within this theory. They could either lack activating ligands or express inhibitory ligands (86).

Natural Killer cells and Dendritic cells - cross relationships

Initially noticed for their ability to act as a first line of defense in the innate immune response, NK cells were later observed to exhibit collaboration behaviors and co-activating relationships with dendritic cells (87) (88). NK cells have also been described as regulators of the DC population by inducing their cellular death (89). These collaborative behaviors are most often observed in lymph nodes and can go two ways, depending on the situation.

In the event of tumorous or damaged cells being detected by NK cells, the latter express TNF-α and IFN-γ stimulating the activation of nearby dendritic cells. Newly activated DCs then take on their role as promoters of the inflammation, and express a range of cytokines (IL-2, -12, -18, -15, IFN-α & IFN-β) that enhance the activity, cytokine secretion, and cytotoxicity of NK cells in a positive loop. In parallel, activated DCs promote the development of an antitumoral response by performing their role of T-cell recruiters. The result of this collaboration is the quick promotion of an adaptive immune response following the early detection of tumor cells by NK sentinels.

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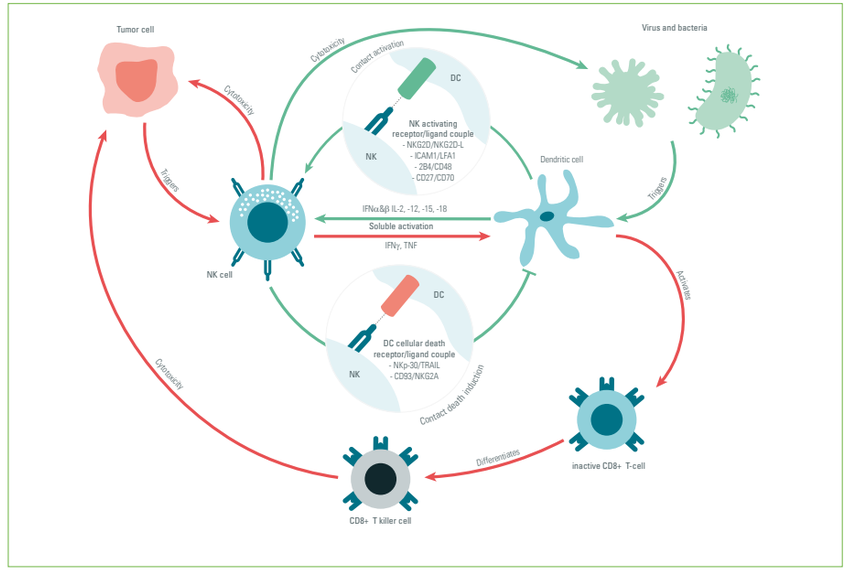
INNATE IMMUNITY

Innate Immune Cell Types

In the event of an infection by a foreign pathogen, DCs are likely to encounter and capture them sooner than NK. When this happens, DCs express the same cytokines as mentioned earlier, which initiates the same activation loop in NK and promotes their ability to destroy the pathogen because of an increased cytotoxicity.

This crosstalk between NK cells and dendritic cells enables them to share with each other the activating signals they receive from their respective different sets of receptors, making them complimentary and promoting quick and efficient recruitment of both types in tumor and infectious disorders alike.



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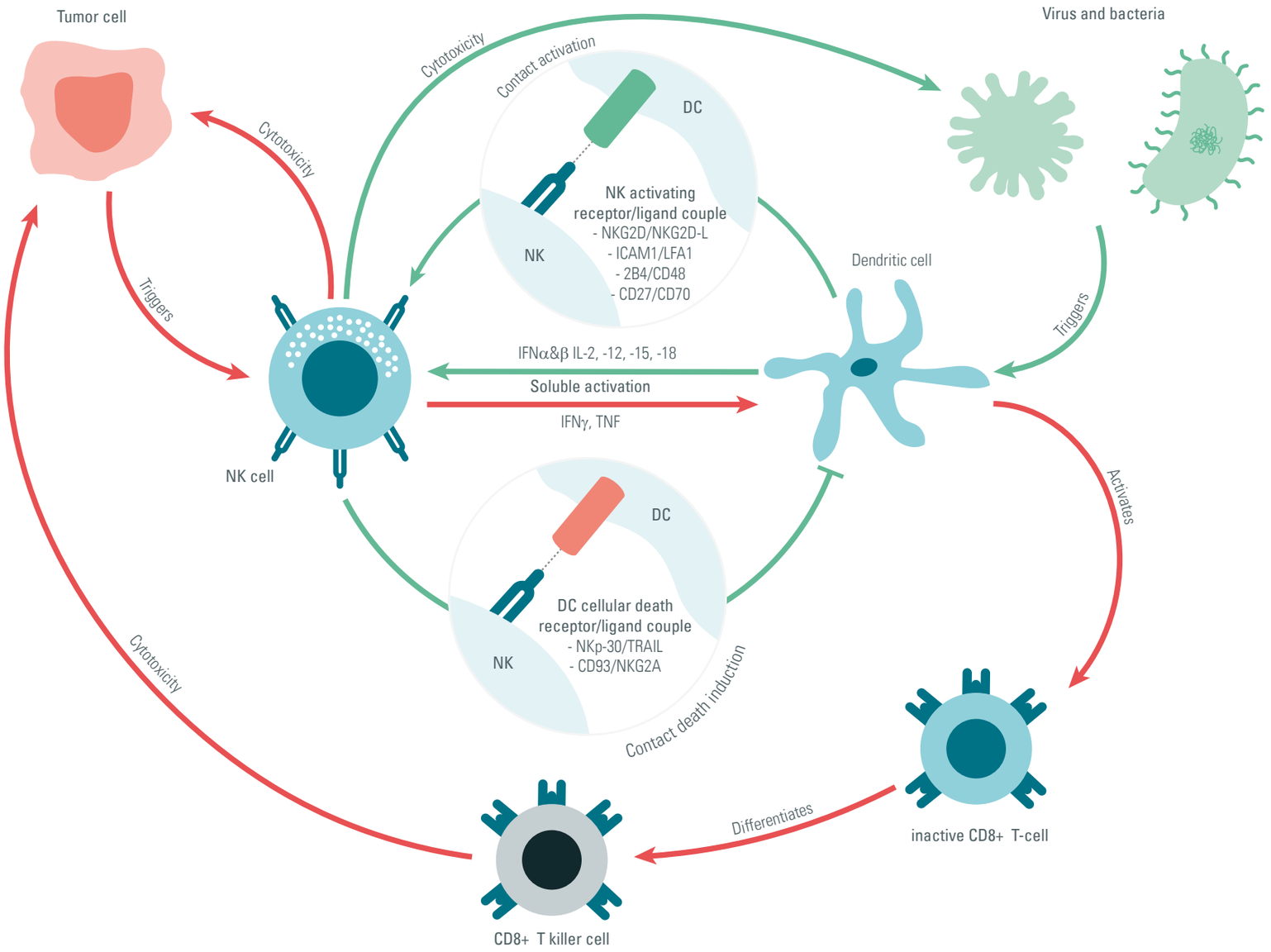

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ADAPTIVE IMMUNITY

B lymphocytes

BCR

B lymphocyte B antigen receptors are called BCR (B-cell receptors). Every single B-cell clone expresses a unique BCR out of the 108 possible combinations allowed by the random recombination of multigenic family (VDJ genes) segments in the genome of stem cells. This operation is processed in the bone marrow as cells mature. At the end of the maturation process, BCRs are tested against self to eliminate or inactivate B-cells that have randomly expressed autoreactive receptors.

BCRs are **membrane immunoglobulins** comprised of two heavy chains (50kDa each) and two light chains (25kDa each) that respectively feature 4 and 2 immunoglobulin domains. The variability and antigen-specificity properties of the receptor are carried by its variable regions, which result from the association of both heavy and light variable domains in what are called the epitopes of the receptor. More precisely, each variable domain of the BCR carries 3 hypervariable regions (HV) that are responsible for its antigen-related properties. The BCR receptor is associated in a BCR complex with the **heterodimeric CD79** protein, which carries two ITAM domains and is responsible for transducing antigen-binding signals inside the cell.

Depending on the naïve or activated state of B-cells, BCRs can either be transmembrane or soluble immunoglobulins, in which case they are referred to as **antibodies**. The switch from transmembrane to soluble immunoglobulins is achieved through an alternative splicing step that removes the hydrophobic transmembrane portion of the receptor when B-cells differentiate into plasmocytes or plasma cells.

As long as B-cells remain in a naïve state, the BCR they express are immunoglobulins of the M or D classes. However upon activation, B-cells proceed to switch their immunoglobulin class from IgM and IgD to IgG, IgE, IgA, and so on.

BCR signaling

The initiation of BCR signaling involves **Lyn** and **Syk**, part of the Src family kinases. Similarly to TCR signalization, antigen binding by the receptor opens the ITAM domains carried by the CD79 heterodimer to **Lyn** phosphorylation, but also BIK and Fyn. This turns them into docking sites for the recruitment and phosphorylation of **Syk**, and eventually, **SLP65** phosphorylation. This leads to the recruitment and phosphorylation of **BTK** at Tyr551 and in turn, BTK autophosphorylates at Tyr223 for its full activation. Phosphorylated

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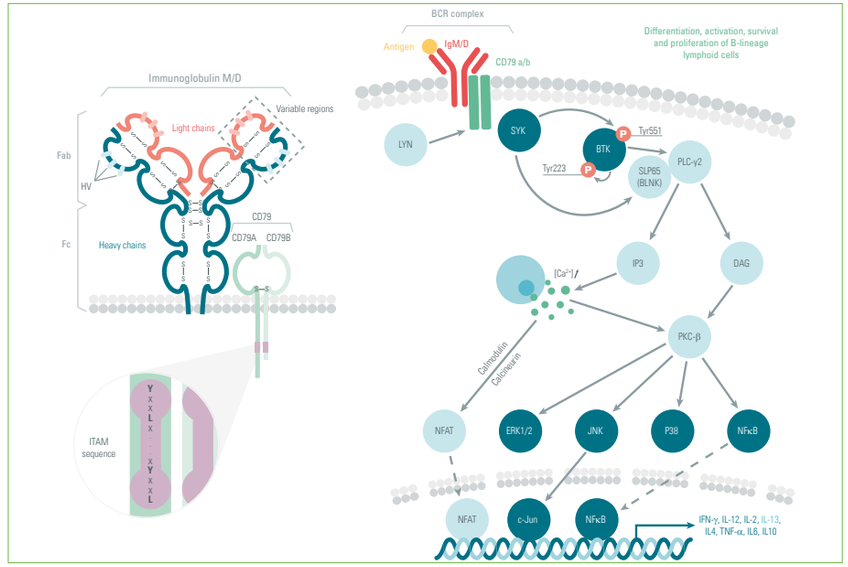
ADAPTIVE IMMUNITY

B lymphocytes

BTK and SLP65 contribute to phospholipase C gamma II **(PLC-γ2)** activation, which then results in inositol phosphate 3 **(IP3)** and diacylglycerol **(DAG)** accumulation. The first promotes the activity of transcription factor NFAT via an IP3R-mediated intracellular calcium increase which binds calmodulin to activate calcineurin, while **DAG** activates protein kinase C beta **(PKC-β)** in presence of calcium, promoting the accumulation of signaling protein, kinase, and transcription factors **ERK1/2, P38, JNK**, and **NF-κB**. This results in the expression of multiple inflammatory cytokines and growth factors including IFN-γ, TNF-α, IL-12, IL-2, IL-13, IL-4, IL-6, and IL-10.



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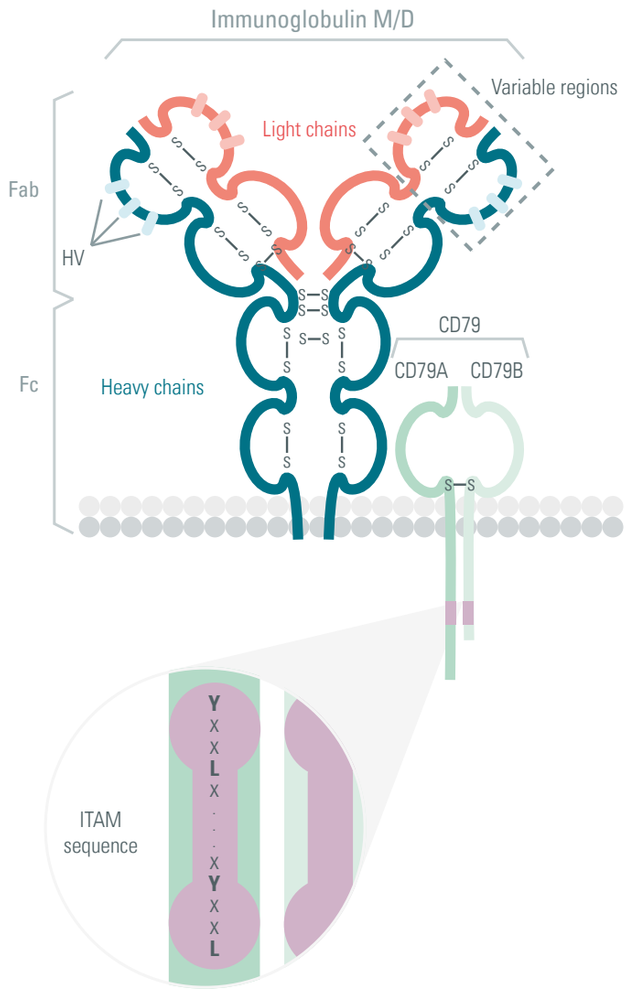

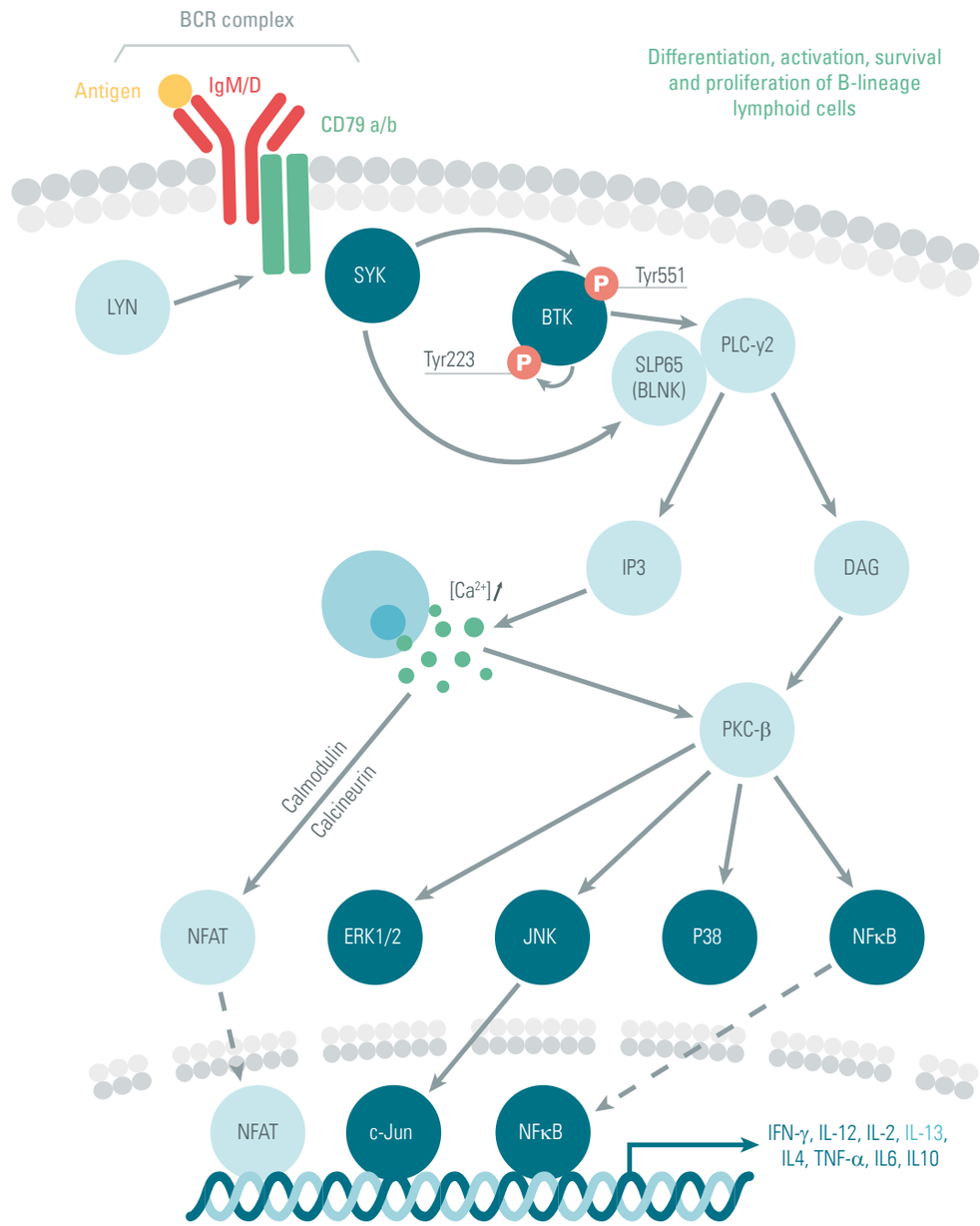

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ADAPTIVE IMMUNITY

B lymphocytes

B CELLS

B lymphocytes are an immune cell type of lymphoid lineage that are found as lymphoid progenitors in the bone marrow and account for 1-7% of leucocytes (29).

They exist in three types: naïve, active/ secreting antibodies (plasmocytes), and memory B-cells. **Naïve B-cells** account for 60-70% of B-cells and mostly express BCRs in the form of IgM, but also a minor fraction of IgD. **Memory B-cells** account for 35-40% of peripheral blood B-cells. They are long-life differentiated B-cells that express soluble immunoglobulins of all classes, especially IgG and IgM-D. Their role consists in preserving the antigen-specificity developed during the immune response to enable a faster and more intense response in the case of a re-infection. **Plasmocytes** are short life differentiated B-cells which account for a highly variable portion of B-cells depending on the immune response level and stage. They express the same immunoglobulins as memory B-cells.

Activation and differentiation

B-cells most commonly activate by binding **thymo-dependent antigens**. This activation mechanism requires three signals, and is initiated when a naive B-cell's BCR matches an antigen, either in circulation or presented by a DC or other antigen presenting cell. The naïve B-cell endocytes the antigen whole with the BCR, and breaks it down to present fragments of it on its MHC II (see Type II major histocompatibility complexes). This first signal is followed by the upregulation of CD40 and CD80 at the B-cell's membrane. The activated B lymphocyte then migrates to the lymphoid organs and presents the antigen fragments to helper T-cells. If a helper's TCR matches the fragments, the two cells interact and co-stimulate each other via the CD40/CD40L and CD80/CD28 couples. This second signal triggers the expression of cytokine receptors and cytokines in the B-cell and helper T-cell respectively. Finally, the third signal is provided by cytokines released by the helper T-cell and triggers the complete activation of the B-cell.

Upon complete activation, B-cells proliferate and a portion of them differentiate into plasmocytes that start secreting IgM (and low IgD). The rest of them migrate deeper into lymph nodes and hypermutate to increase the affinity of their BCRs for the antigen. The resulting new receptors are tested against a collection of potential antigens expressed and presented by specialized follicular cells of the node, then again by T-cells for autoreactivity. The surviving ones are allowed to switch their immunoglobulin class from IgM to mostly IgG, then proliferate and differentiate into plasmocytes and memory B-cells.

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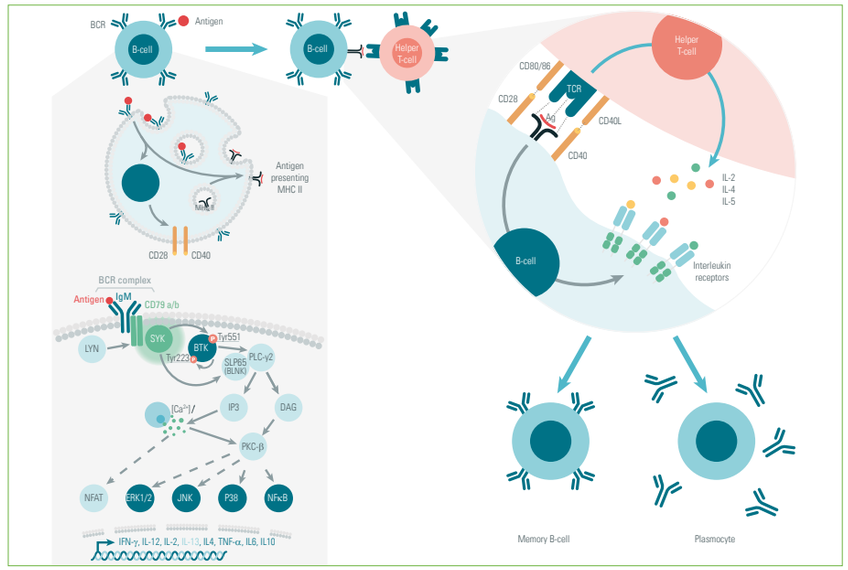
In the event of a **thymo-independent antigen** activation, B-cells can differentiate without the support of a helper T-cell. This occurs when the antigen bound exhibits repetitive patterns (bacterial walls, viral capsids, carbohydrate, lipopolysaccharides, etc.) that activate numerous BCRs on the same B-cell. In such situations the resulting BCR-induced signal is very strong, and overrides the need for a helper. However, without the contribution of a helper T-cell, activated B-cells do not switch immunoglobulin classes and only secrete IgM antibodies.



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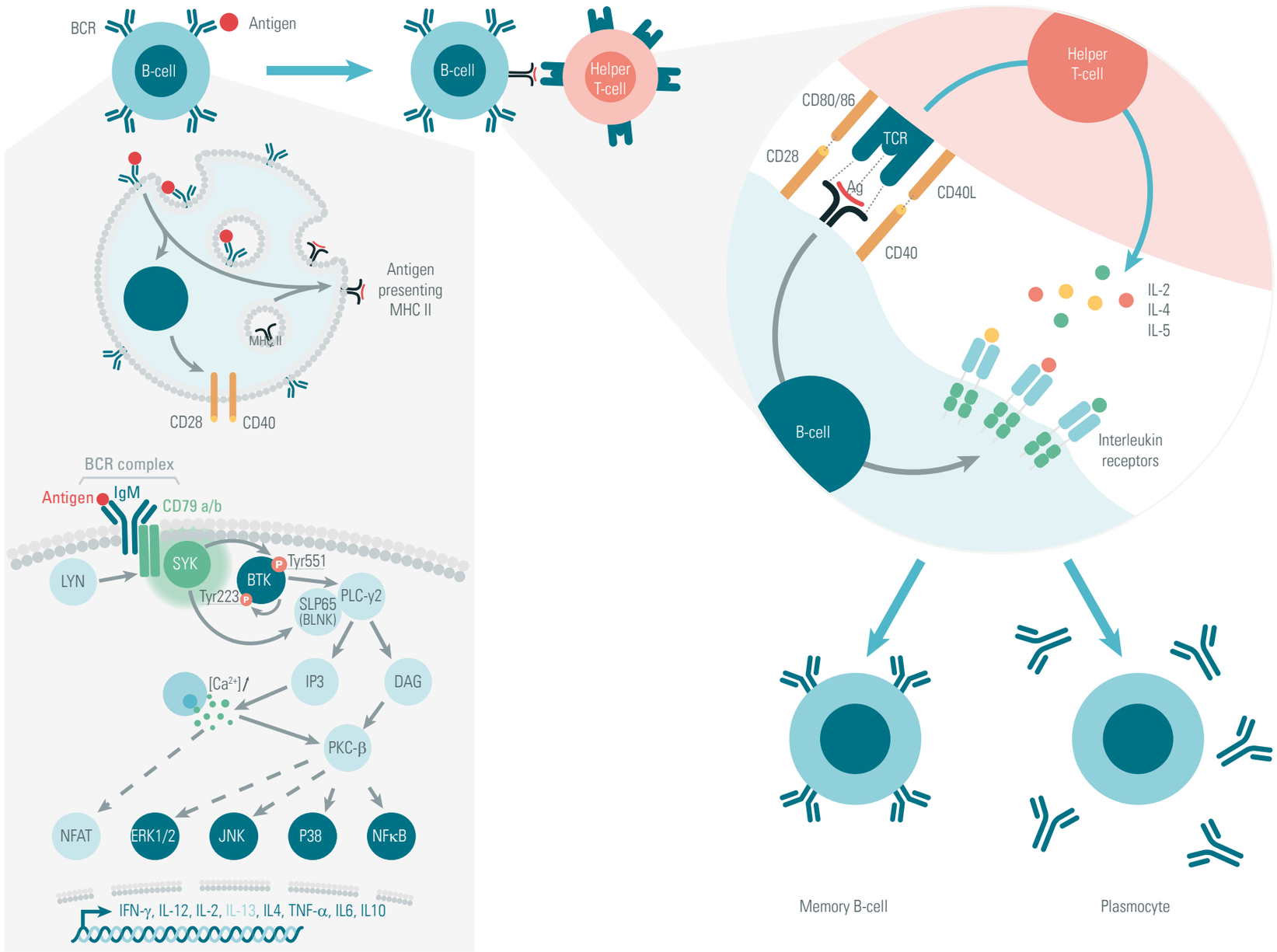

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ROLES OF ANTIBODIES

Once expressed toward a specific antigen by B-cells differentiated into plasmocytes, antibodies perform a collection of roles that are grouped under the term **humoral-mediated immune response**. These functions include strategies that impair pathogens, remove their pathogenicity, promote their removal, or enhance and facilitate the activity of the professional phagocytic and lytic cells.

Opsonization

Antibodies act as opsonins and tend to coat pathogens. This neutralizes them and makes them easy targets for professional phagocytic or lytic cells, which are driven to them by their Fc receptors (RFc) that bind the Fc segments of antibodies. In such situations, the adaptive immunity of antibodies drives the innate defensive abilities of phagocytes to pathogens. Opsonization is at the root of antibodies' functions and is responsible for all their other roles.

Pathogen neutralization

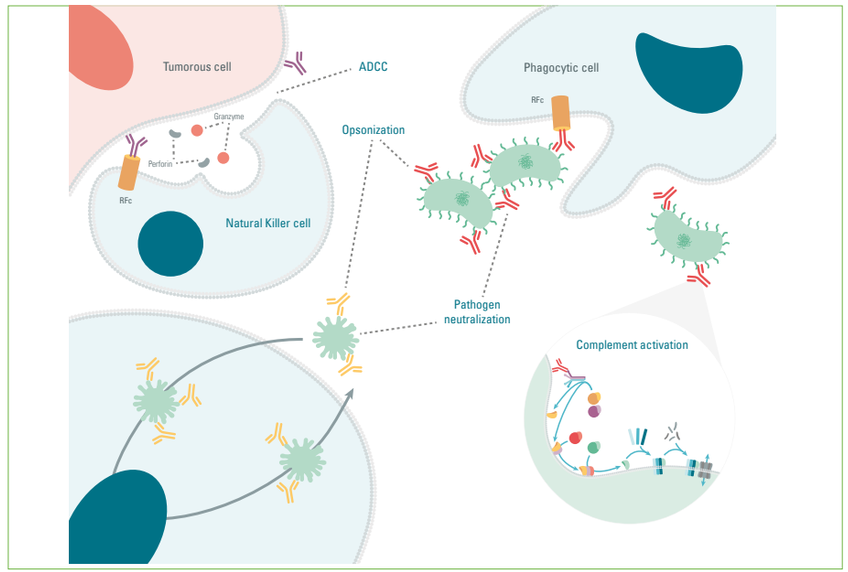
Antibodies binding to antigens can neutralize their pathogenicity by removing their ability to catalyze reactions or to signal through receptors in the case of toxins, or by promoting their aggregation and impair their adhesion to cell membranes for bacteria. Depending on their specificity they can tackle a virus life-cycle at any point, including blockade of their attachment ligands or cell-entry mechanisms, intracellular immobilization pre-disassembly of capsids, inhibition of new viral particle assembly, or even promotion of ubiquitination and subsequent lysosome lysis of any viral component.

Antibody-Dependent Cellular Cytotoxicity (ADCC)

ADCC is a phenomenon that occurs when antibodies binding to a pathogen drive the unspecialized cytotoxic or lytic killer cells to it. These cells bind antibodies in the same way as their phagocytic colleagues, but deliver a different cellular death to their target. The two antibody subsets IgG1 and IgG3 are the most effective in driving this process, even though IgG3 only have a 5-day half-life which makes them less relevant in the long term.

Complement activation

Antibodies are key factors in the activation of the complement, as they initiate its classical activation pathway at the surface of pathogens by recruiting the ty of immune cells at the site. See chapter "Innate Immunity": "The complement system", page 20.



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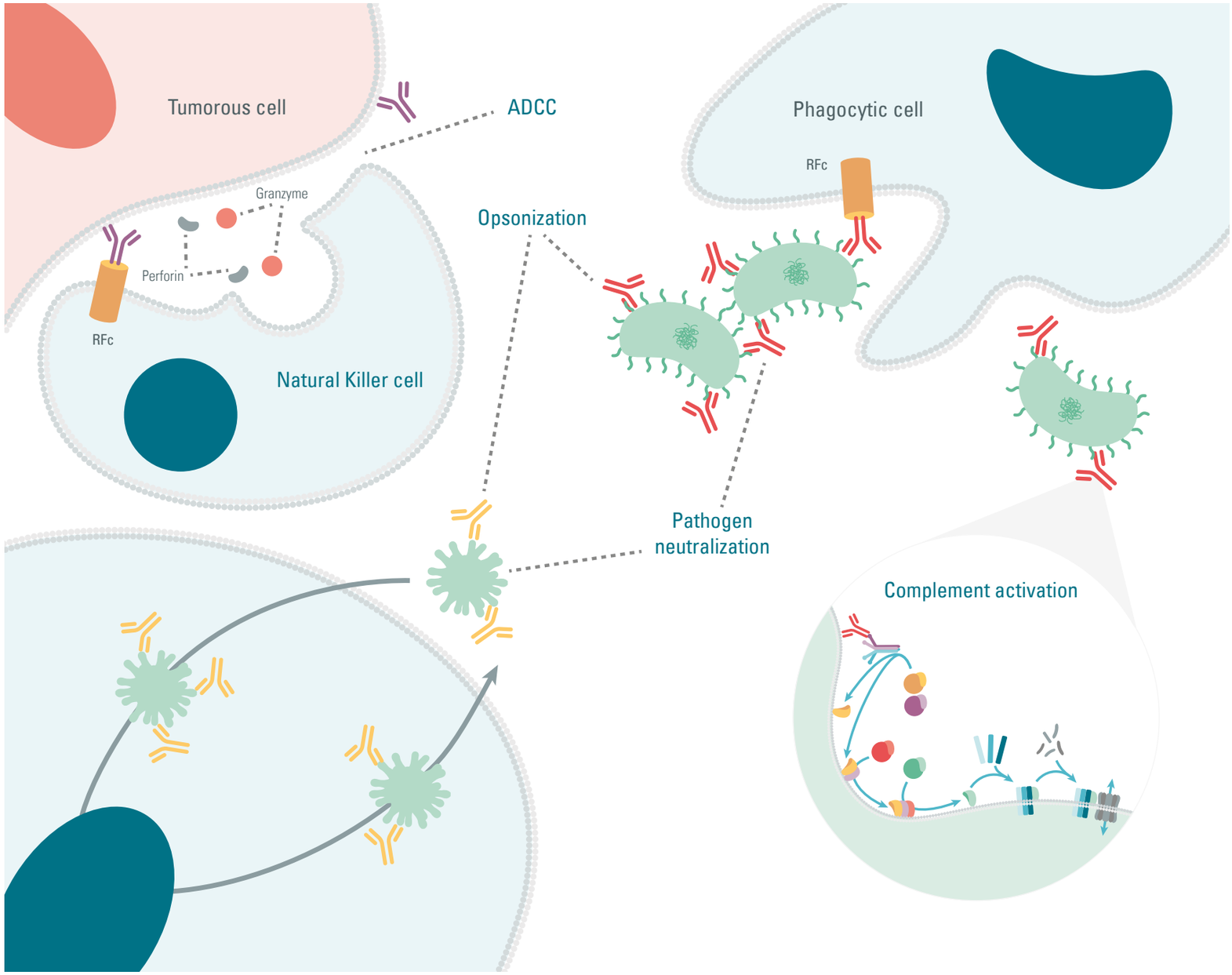

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T lymphocytes

TCRS

T lymphocyte antigen receptors are called TCRs (T-cell receptors), and are associated in TCR complexes with a CD3 protein and a homodimer of zeta chains. The resulting complex is a receptor with degenerate specificity likely to bind a wide variety of antigenic peptides (roughly 5. 10^5) and is MHC restricted, meaning it requires antigens to be presented by an MHC from the same organism (histocompatible).

The **TCR part** of the receptor is comprised of one α-globulin and one β-globulin chain, each exhibiting a constant and a variable domain. The latter's variability is itself carried by three hypervariable regions (HV) that make up the specificity of the receptor. This variability results from the random recombination of genic segments during the development (10^{10} combinations). **CD3 protein** is a 4-transmembrane non-variable chain complex made of two ε chains, one δ chain, and one γ chain (CD3εγ / CD3εδ heterodimer), which contributes to the complex stability and signal transmission after TCR antigen recognition. Finally, the **zeta homodimer** is mostly intracellular and is responsible for the signal transduction inside its host, thanks to the 6 ITAM (Immunoreceptor Tyrosine-based Activation Motif) sequences it carries.

TCR signaling

Upon binding an MHC-presented peptide, the TCR complex enters a conformational state that leaves its intracellular ITAM domains open to phosphorylation from **Lck**. Phosphorylated ITAMs then recruit **ZAP-70**, which activates and phsophorylates a number of partners including CD28, **LAT**, and **SLP-76**. The latter is recruited at the membrane by phosphorylated LAT, where it promotes the activation of phospholipase C gamma I (**PLC-γ1**) whose activity cleaves Phosphatidylinositol-4,5-bisphosphate (PIP2) to produce diacylglycerol (**DAG**) and inositol phosphate 3 (**IP3**).

IP3 diffuses rapidly to activate calcium channel receptors on the endoplasmic reticulum, inducing the release of Ca2+ into the cytosol. This calcium binds calmodulin, leading to the activation of calcineurin which activates the transcription factor **NFAT**. Meanwhile, DAG promotes the activation of **ERK1/2** via the MAP kinase axis (Ras-Raf-MEK1/2) which results in transcription factor **Fos** activation.

Simultaneously with the Antigen/TCR-induced signaling, T-cell activation is achieved by stimulation of its **CD28** by the CD80/86 of the dendritic cell presenting the TCR with an antigen. This leads to the activation of **PI3K** which phosphorylates PIP2, to produce Phosphatidylinositol-3,4,5-trisphosphate (PIP3), ultimately leading to the activation of Phosphoinositide-dependent kinase-1 (**PDK1**) and the consequent promotion of signaling proteins **AKT**, **mTOR**, **JNK**, and **NF-κB**.

The resulting transcription factors (NFAT, NF-κB, FOS, JNK) activate the transcription of sets of genes, including IL-2 and IL2R, which promote long-term

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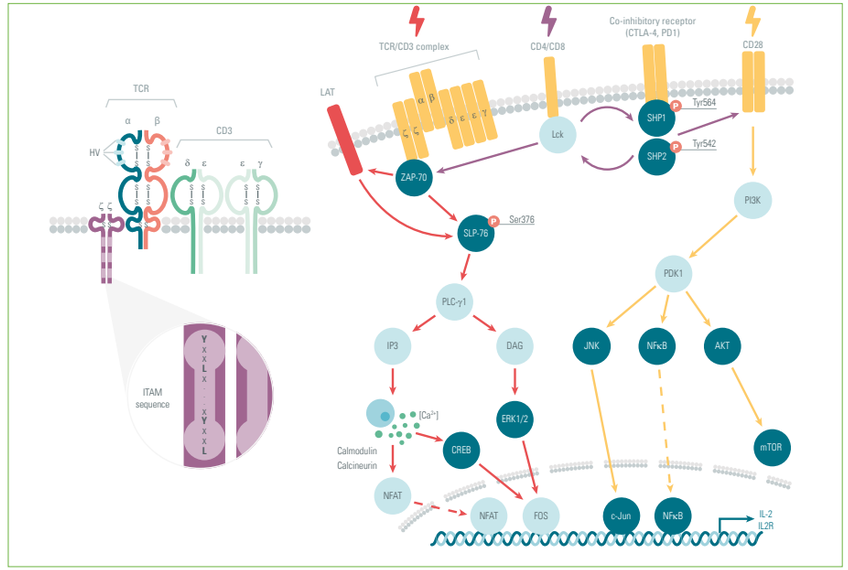
proliferation of activated T cells.

The overall signaling ability of the TCR pathway is regulated by the co-inhibitory receptors **CTLA-4** and **PD1**, which recruit and activate SHP1 and 2 whose phosphatase activities impair the early roles of LcK and CD28 in T-cell stimulation.



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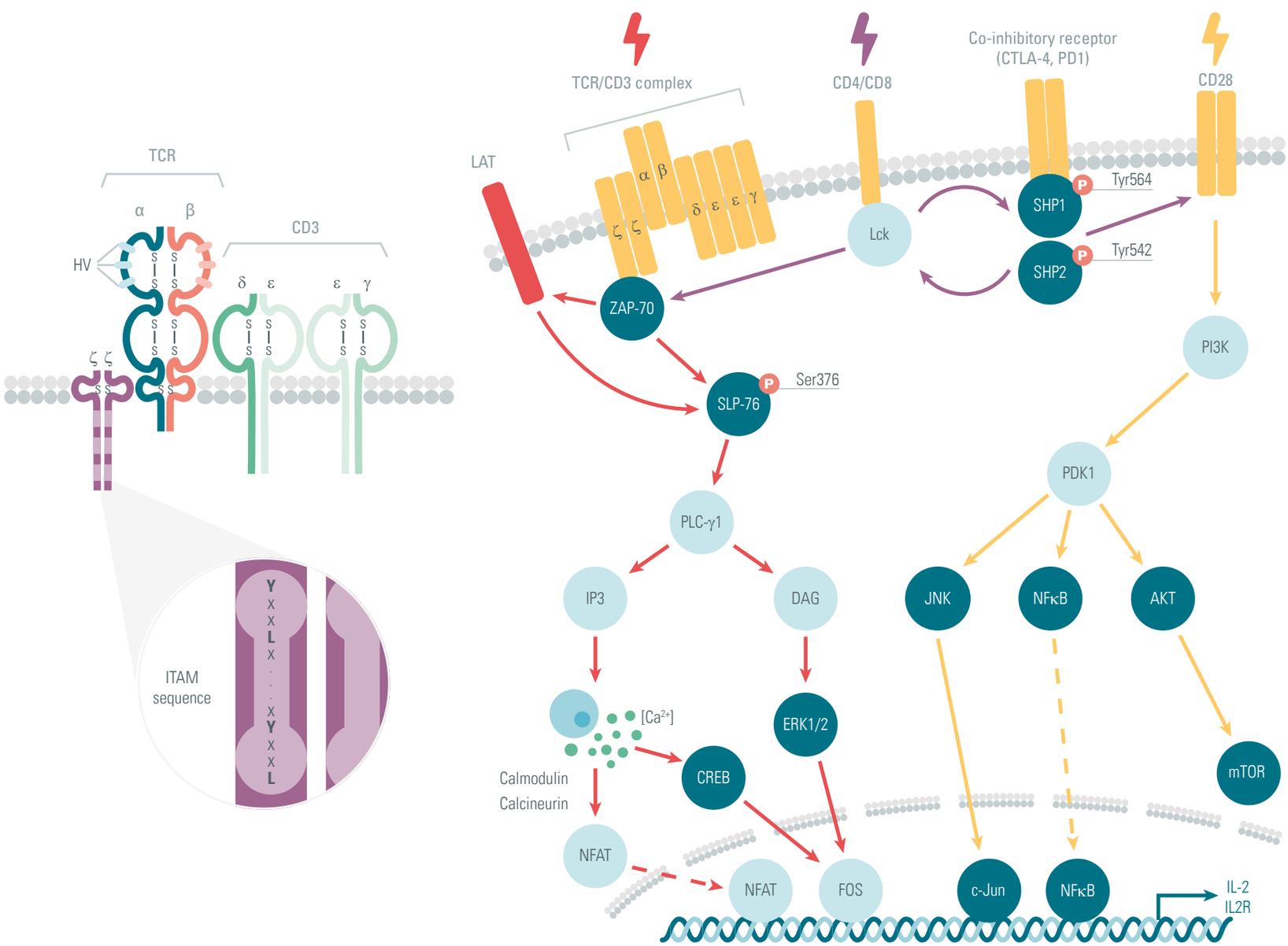

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T lymphocytes

T-LYMPHOCYTES

T-lymphocytes are a family of immune cells of lymphoid lineage characterized by their TCR receptors and CD4 or CD8 markers, accounting for 7 to 24% of leukocytes (29). These markers contribute to the interaction of T-cells with MHC II and I respectively. Mature T-cells only express one of these marker types, which greatly influence their role in the immune response once activated. **CD4+ T-cells** differentiate into helpers and regulators that activate, monitor, and downregulate other immune cells, while **CD8+ T-cells** evolve to become cytotoxic killers that target and eliminate infected, damaged, or tumorous cells (See related chapters).

T-cells are bone-marrow-borne as pre-thymocytes from lymphoid progenitors before migrating to the thymus and maturing into thymocytes. They then undergo a rigorous maturation process called the thymic selection, that ensures their TCR receptors recognize both MCH of their host and none of its self-protein. This selection is so stringent that a mere 5% of thymocytes make it through before being released in circulating blood.

Development and maturation

Thymocytes coming from the bone marrow do not express CD markers yet. Upon migration to the thymus, they go through a genetic reorganization that modifies their TCRs and expresses both CD4 and CD8 markers. They later proceed to eliminate one of the markers and express only the other, but this process is not described.

The first selection step takes place in the thymus, where thymocytes are tested against epithelial thymus cells expressing both MHC. This step ensures the future MHC restriction and recognition of self of the T-cells. During the second selection step, Thymocytes are tested against dendritic cells expressing all types of proteins of self on their MHC. This ensures the elimination of autoreactive future T-cells.

Activation and differentiation

T-cells recognize antigens with their TCR if and only if presented on the MHC of another cell of self (recognition is restricted to the MHC). Upon such presentation, a T-cell either recognize both antigen and MHC and undergoes its activation process, or it does not and remains inactive. For both CD4+ and CD8+, complete activation leads to effector T-cells which take on the role of helpers or regulators for CD4+ and cytotoxic killers for CD8+ cells (pages 52-56). A fraction of activated T-cells also differentiates into long-life memory T-cells, to preserve the antigen specificity over time and enable a more effective response in the event of a re-infection.

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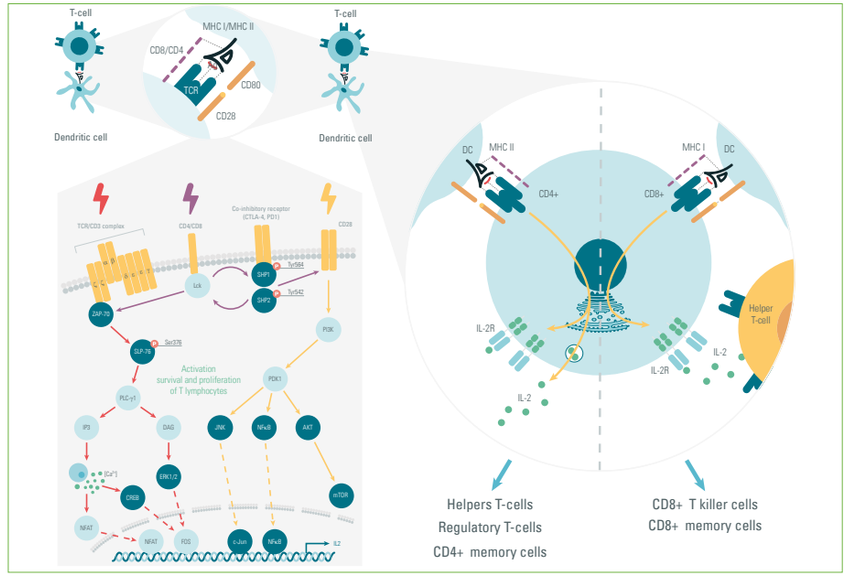
The exact mechanism and partners involved differ between CD4+ and CD8+ T lymphocytes but include three independent signals for both. For **CD4+ T-cells**, the antigen is presented on the **MHC II** of an antigen-presenting cell that comes in contact and anchors onto the T-cell. The TCR and MHC II interact as the T-cell's CD4 bind the MHC II, which sends the first signal through the ZAP-70 related pathway (page 48: TCR). Co-stimulation molecules CD28 and CD80/86 on the T-cell and dendritic cell respectively constitute the second signal, and promote the expression of IL-2 ad IL2R by the T-cell. The last signal is mediated by the IL-2 in an autocrine activation of the cell.

For **CD8+ T-cells** the antigen is presented on the **MHC I** of an antigen-presenting cell (dendritic cell). The first two signals are quite like those of CD4+ T-cell activation, except for CD8 binding the MHC I instead of CD4 binding the MHC II. The last signal however is not an autocrine activation but requires the mediation of a helper T-cell that provides the CD8+ T-cell with IL-2 to complete its activation.



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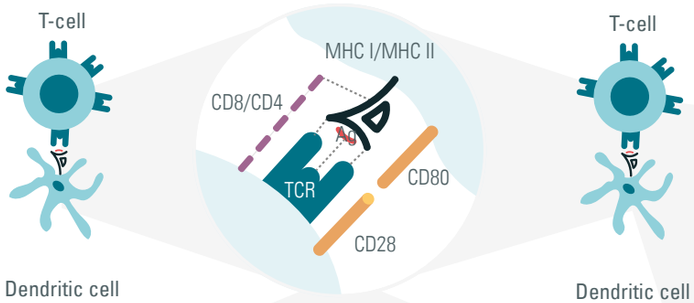

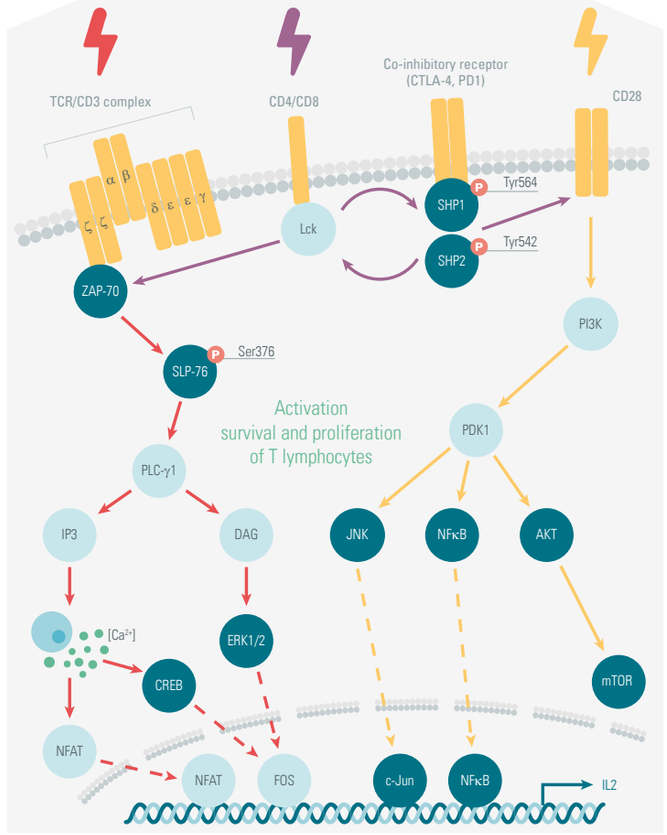

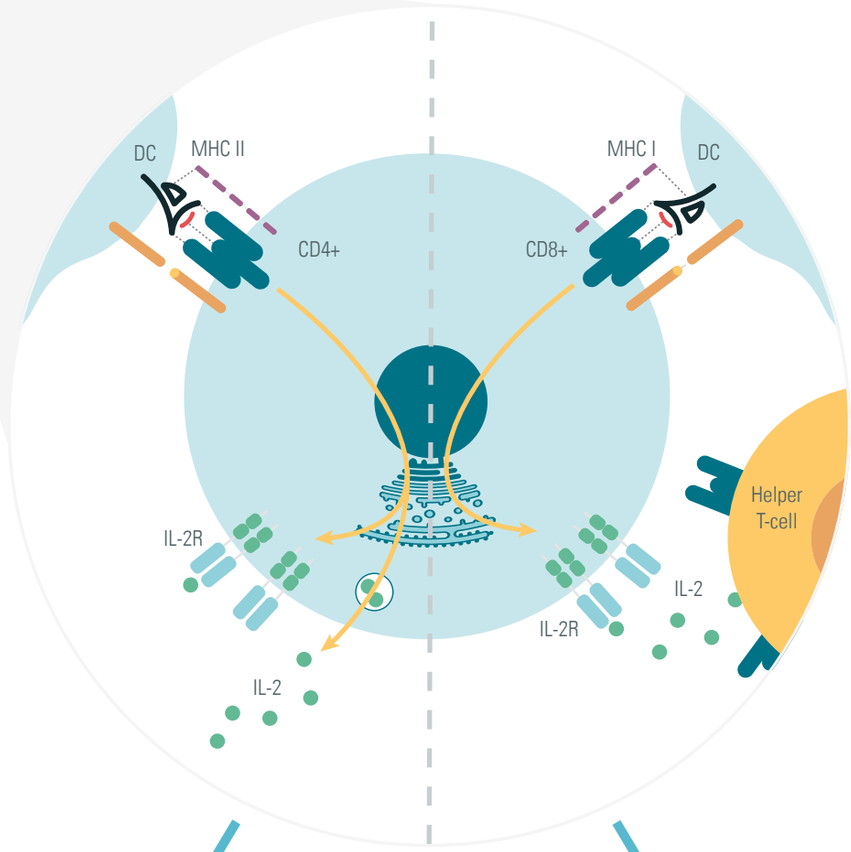

Helpers T-cells
Regulatory T-cells
CD4+ memory cells

CD8+ T killer cells
CD8+ memory cells

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T lymphocytes

CD4+ HELPER T LYMPHOCYTES

Helper T-cells are a family of differentiated effector T-cells whose activity supports, activates, and promotes the actions of other immune cell types. Their contribution to the development of an adaptive and humoral immune response is critical, as antibody-secreting B-cells and cytotoxic CD8+ T-cells are unable to perform the full extent of their missions without their support. They exist in several subtypes with different phenotypes and roles: **Th1**, **Th2**, **Th17**, **Th9**, **Th22**, and **T follicular helper** (**Tfh**). Polarizations into subtypes are carried out following exposure to specific cytokine mixes, and guided by transcription factors. In the case of Th1, Th2, and Th17, such factors are unique and considered as master transcription factors TBET, GATA3, and RORγt respectively (90) (91) (92).

Helper subtypes

Th1 differentiate in presence of IL-12 and promote the activity of phagocytic cell macrophage through a consistent expression of IFN-γ. They also play a key role in adaptive cytotoxicity, by providing the IL-2 necessary for the activation of CD8+ T-lymphocytes.

Th2 arise from stimulation by IL-4, and enhance pathogen clearing through stimulation of eosinophils, basophils, and mastocytes with IL-4, IL-5 and IL-13. They also contribute to the humoral response by providing IL-2 for B-cell activation, and sustain the overall inflammation with the pro-inflammatory IL-6. It is worth noting that they mediate their own differentiation in a positive IL-4 feedback loop.

Th17 are induced by IL-6, IL-21, and TGF-b, and are potent pro-inflammatory cells that drive neutrophils and macrophage to infection sites with their secretion of IL- 17A, IL-17F, IL-21, IL-22, and CCL20. Due to their inflammatory functions, defects in their behavior are suspected of contributing to many autoimmune diseases (93).

Th9, Th22, and Tfh are recent additions to the family of described helpers. Consequently, their roles, polarizing cytokines and transcription factors remain uncertain and continue to be discussed. **Th9** are described by their IL-9 expression. This phenotype occurs in presence of IL-4 and TGF-β, but can also be promoted by IL-2, IL-1 family, IL-33, and IL-25, or repressed by IFN-γ and IL-27. Unlike Th1, -2, and -17, Th9 differentiation is suspected to rely on several transcription factors such as IRF4, PU.1, and GATA3. It has been suggested that Th9 plays a role in stimulating Treg suppressive functions and Th17 proliferation (94).

Th22 secrete IL-22 and polarize in presence of IL-6 and TNF-α, which signal through the transcription factor AHR (Aryl Hydrocarbon Receptor) to drive their phenotype by promoting and inhibiting IL-22 and IL-17 expression respectively. Il-22 targets specific interfacing cell types, such as keratinocytes, hepatocytes, fibroblasts, and epithelia, and exerts different effects (e.g. production of survival or anti-microbial proteins, increased cell mobility, reconstruction, or proliferation) (95).

Polarization of T-cells into **T follicular helpers** (Thf) is a multistep process. Though it lacks complete description, it is known to occur in the absence of other helper polarization signals and in presence of IL-6, IL-21, IL-23, and other potential candidates (IFNs, TGF-β). BC16 has been identified as an essential transcription factor for the phenotype of Tfh, but some others have been suggested (STAT4 and TBET, c-Maf, IRF4, Batf). Thf contributes to B memory lymphocyte differentiation or immunoglobulin class switching, and contributes to the hypermutation steps B-cells undergo upon activation (96) (93).

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DIFFERENTIATION CYTOKINE COCKTAIL	HELPER T-CELL	MAIN CYTOKINES AND TRANSCRIPTION FACTORS EXPRESSED		TARGETED CELLS	ROLES IN IMMUNE RESPONSE
IL-12	 Th1	IFN-g IL-2	STAT4 **TBET** LT-a	Macrophages CD8+ lymphocytes	Response to intracelullar pathogen and defects trhough macrophage (IFN-g) and CD8+ T-cell activation (IL-2)
IL-4	 Th2	IL-4 IL-5 IL-6 IL-13	STAT6 **GATA3**	B lymphocytes Eosinophils Basophils Mastocytes	Response to extracelullar pathogen and defects through B-cell activation and overall killing cell stimulation. Sustain inflammation.
IL-6 IL-21 TGF-b	 Th17	IL-17 IL-21 IL-22 IL-1	STAT3 **RORgt**	Neutrophils	Promote myeloid cell activity (DCs, NK, granulocytes, macrophages). Promote and sustain inflammation.
IL-4 TGF-b	 Th9	IL-9	STAT6 PU.1 IRF1 IRF4 BATF FOXO1	Mastocytes Lymphocytes	Recruit lymphocytes and mastocytes.
IL-6 TNF	 Th22	IL-22 TNF-a	STAT3 AhR	Keratinocytes Epithelial cells Hepatocytes	Promote inflammation. Observed to enhance autoimmune disease psoriasis.
IL-6 IL-21 Type I IFNs ?	 Thf	IL-21 IFN-g IL-4 IL-9	STAT3 STAT4 BCL6	B lymphocytes	Promote B-cell activation, class switching and germinal center formation for hypermutation

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T lymphocytes

CD4+ REGULATORY T LYMPHOCYTES

Regulatory T-cells or T-reg are a subtype of effector T-cells whose phenotype and role make them down-regulators of the activation and proliferation of other CD4+ or CD8+ effector T-cells. It has been reported that they could also have downregulation behaviors toward B-cells and dendritic cells (97).

The arsenal that helps them in their mission of immune downregulation includes the secretion of immunosuppressive mediators, contact-dependent modulation of immune cells, cellular death induction, cytokine starvation, and dilution of immune cell populations (98). Their proliferation and function are sustained by IL-2, which they do not express but is however produced by other effector T-cells. This results in a regulating loop that ensures T-reg deactivation and decrease as they downregulate effector T-cells, effectively starving themselves in IL-2. (99)

Characterization

T-regs are usually split between those occurring naturally and those which are induced. **Natural T-regs (nTreg)** are characterized by markers CD4 and CD25 (IL-2R subunit) (100) and master transcription factor FoxP3 (101) (102). They are known to carry checkpoint inhibitors, like CTL-4 (CD152) and GITR (Glucocorticoid Induced TNF-Receptor), (103) (104). Natural T-regs arise in the thymus from naïve T-cells differentiating in presence of IL-2, TGF-β and antigen presenting cell stimulation. They fulfill all the usual roles of T-regs, including TGF-β secretion, contact-dependent and cytotoxicity-based inhibitions.

Induced T-reg (iTreg) develop in the periphery from CD4+ FoxP3- T-cells. The most studied are the IL-10-dependent type 1 (Treg 1) which are induced by IL-10. They exhibit low levels of CD25 and CD45B, and lack FoxP3. Due to the absence of that transcription factor, they do not express the full phenotype of T-regs and are incapable of cytotoxicity or contact-dependent inhibition. They do however express high levels of IL-10 and IL-2, which contribute to suppressing CD4+ T-cell proliferation (105) (106).

Regulation strategies

The main suppressive messengers which T-regs **express and secrete** are TGF-β (107) and IL-10 (108). T-regs also strongly promote the immunosuppressive metabolite adenosine (109) by expressing the transmembrane ectonucleotidases CD39 and CD73, which respectively convert ATP to AMP and AMP to adenosine (101) (110).

T-reg **contact-dependent** inhibitory potential is mediated through their transmembrane protein CTL-4, which binds APCs and disrupts their crosstalk with effector T-cells, hence reducing the stimulation of the latter. In such events, CTL-4 binds the B7-1 or B7-2 costimulatory ligands of APCs' CD80 and CD86 respectively (111) (112), resulting in the non-availability of CD80 and CD86 for interaction with effector T-cells via the CD28/CD80-86 crosstalk (113). The exact process of this CTL-4-induced inhibition has not been described, but evidence suggests it involves a removal of CD80 and CD86 from dendritic cells via endocytosis by T-reg (114). CTL-4 also exists as a soluble protein that blocks the DC/T-cell crosstalk by simple binding to CD80 or CD86.

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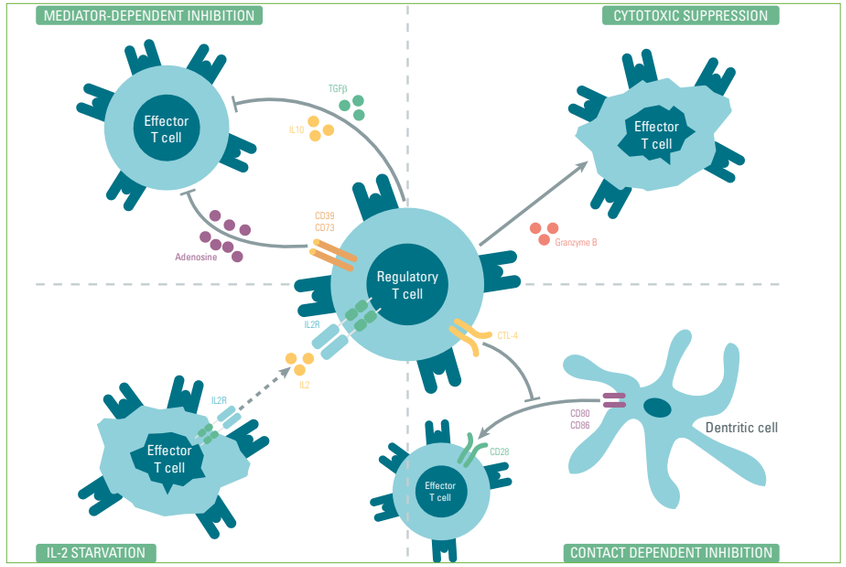
More radically, T-reg directly reduce effector T-cell populations via the cytotoxic granzyme/perforin axis that induces **cellular death** in the same way as their CD8+ cytotoxic cousins (see CD8+ T-cells) (115) (116).

Finally, as stated previously, T-reg consume IL-2 via CD25 which serves as a pump, reducing its availability for effector T-cells, leading them to an anergic state (117). It has been postulated that this **cytokine starvation** strategy could be extended to a global **competition** for growth factors, resources, and the number of interactions APCs can make, which would constitute a passive way for T-regs to limit the number of other effector T-cells the organism can sustain at once.



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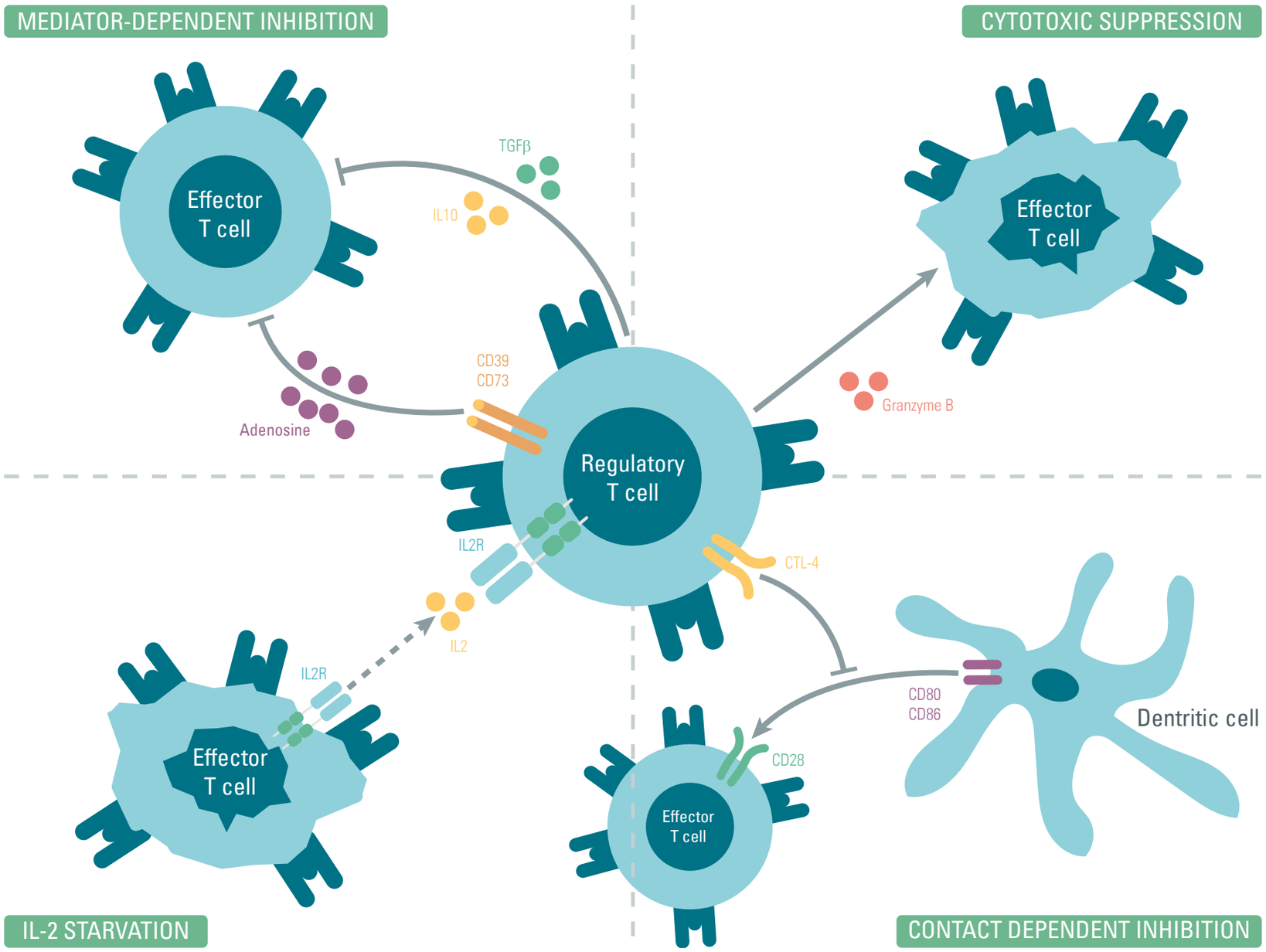

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T-CELL CD8+ CYTOTOXIC

Cytotoxic CD8+ T-cell are a subtype of effector T lymphocytes, whose role in the immune response consists in eliminating cells of self that are tumorous, infected, stressed, or damaged and dysregulated in some other way.

They differentiate from naïve CD8+ T-cells in lymphoid organs through a heavily regulated process that requires three independent signals. First, the T-cell's TCR needs to recognize an antigen presented on the MHC I of APC, most often dendritic cells. This first interaction is mediated via the T-cell CD8 which binds MHC I. The second signal is the DC/T-cell crosstalk in which CD28 interacts with CD80 or CD86 on the dendritic cell surface. Finally, the process cannot be complete without the contribution of IL2 produced by helper T-cells.

After receiving these three signals, the naïve T-cell differentiates into an active cytotoxic killer which eliminates any cells it encounters if their MHC I-presented peptide matches its TCR. Some T-cells also evolve into memory cells.

Mechanism of action

Cytotoxic CD8+ T-cells identify their target thanks to their TCRs that recognize pathogenic peptides presented on cells' MHC I (usually tumor-specific or virus-specific peptides). They then proceed to closely approach their target and destroy it through an induced cellular death process, at the end of which the victim's nucleus is fragmented and cellular content external release is prevented. This step requires a sealed junction or immunological synapse between the T-cell and its soon-to-be victim, as highly toxic and apoptotic compounds are released.

Cytotoxic T-cells' killing abilities rely on three simultaneously-used strategies. The first and most potent one is the **granzyme/perforin axis**, which they share with their CD4+ Treg cousins. They release granules containing perforins and granzymes, which respectively assemble in pore-like structures through the target's membrane and enter those pores to activate pro-caspases that initiate a cellular death cascade.

Another expresses **death ligands** such as FasL that bind death receptors (FasR) on the target's surface. These receptors are usually trimeric and unassembled in the absence of ligands. When one of them is bound, it triggers the trimerization of the complete receptor around its ligand, which promotes the activation of pro-caspases.

Finally, cytotoxic T-cells express **cytokines**, among which TNF-α, which promotes apoptosis through its death receptor TNFR1, much like death ligands do through their own.

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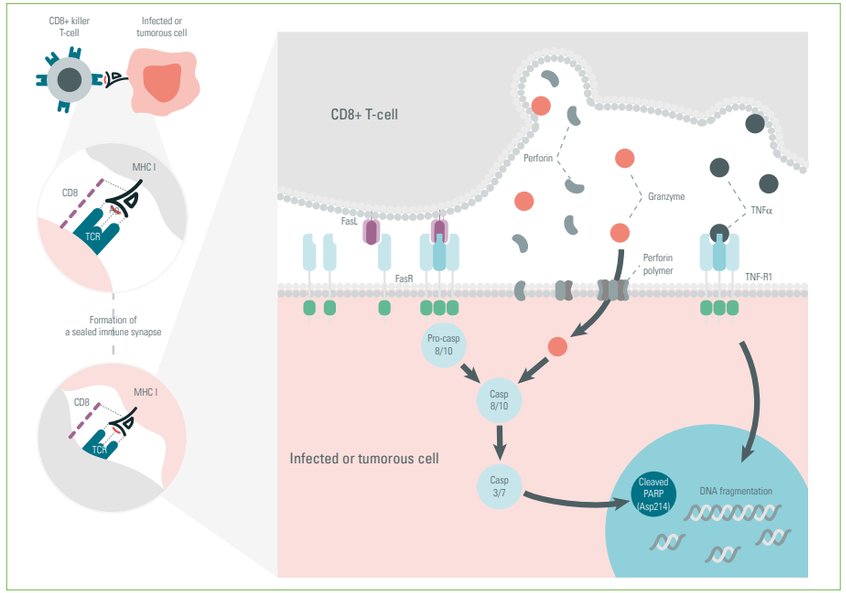
In response to death ligands and the activation of extrinsic or intrinsic apoptosis pathways by cytokines or granzymes, the nuclear enzyme PARP-1, which is involved in the repair of damaged DNA, is cleaved by activated caspases 3 and 7. The cleavage deactivates the enzyme thus impairing its capacity to repair the damaged DNA and allowing for apoptosis to proceed. Cleaved PARP-1 is therefore considered as an essential marker of apoptosis.



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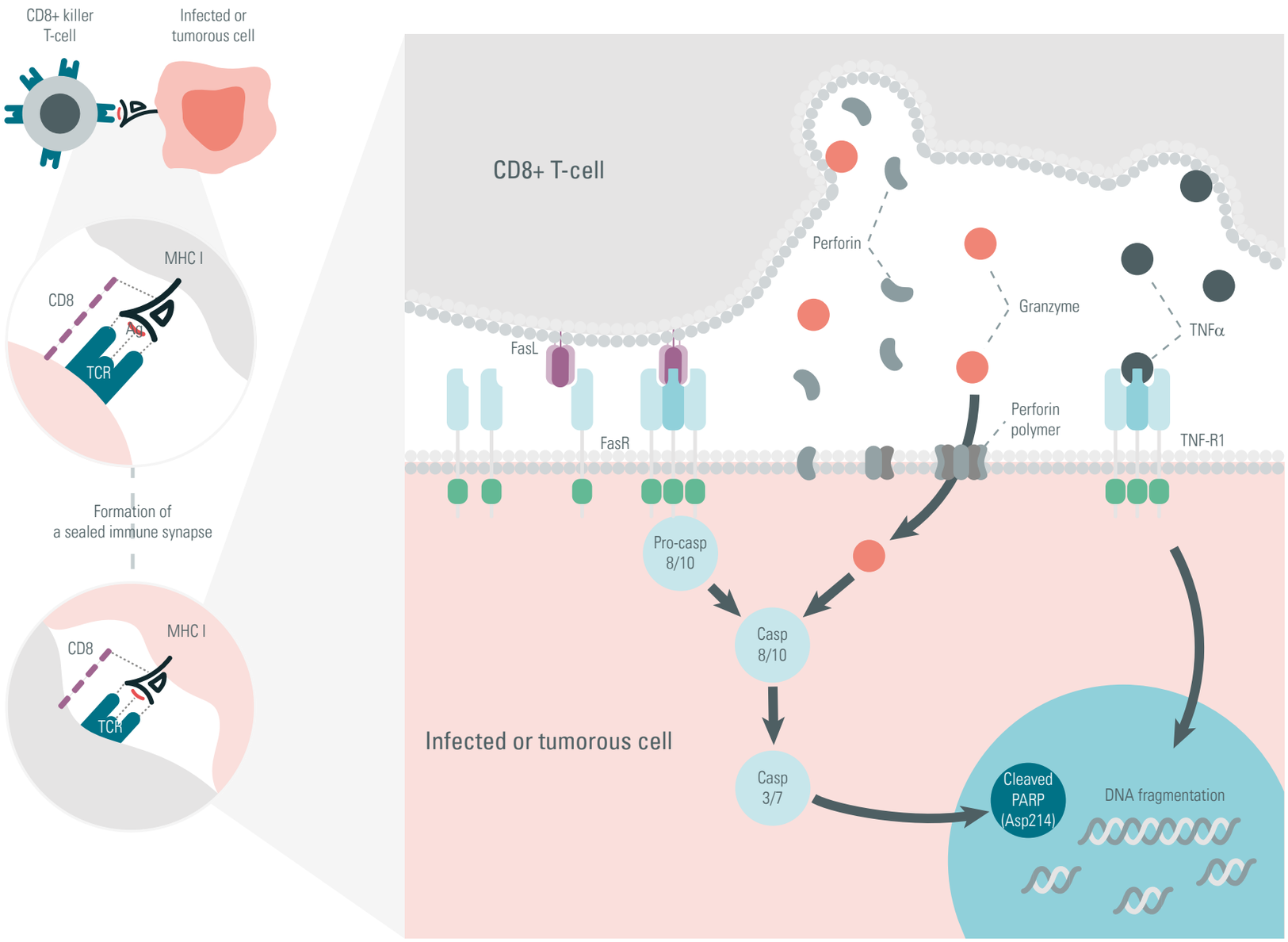

TABLE OF CONTENTS

PerkinElmer® For the Better

WORKS CITED

1. Janeway C.A Jr, Travers P, Walport M, et al. Immunobiology: The Immune System in Health and Disease. 5th edition. New York : Garland Science, 2001. 0-8153-3642-X.
2. Myeloid cell origins, differentiation, and clinical implications. Weiskopf K, Schnorr P.J, Pang W.W, Chao M.P, Chhabra A, Seita J, Feng M, Weissman I.L. 5, October 2016, Microbiology Spectrum, Vol. 4.
3. Hematopoietic Stem Cell: Self-renewal versus Differentiation. Seita J, Weissman I.L. 6, November-December 2010, Wiley Interdisciplinary Reviews: Systems Biology and Medicine, Vol. 2, pp. 640-653.
4. Fiedler K, Brunner C. Mechanisms Controlling Hematopoiesis. [ed.] United Kingdom Charles Lawrie - University of Oxford. Hematology - Science and Practice. s.l. : IntechOpen, 2012.
5. Genetic Control of MHC Class II Expression. Ting J.P, Trowsdale J. April 2002, Cell, Vol. 109, pp. S21-33.
6. Predominant naturally processed peptides bound to HLA-DR1 are derived from MHC-related molecules and are heterogeneous in size. Chicz R.M, Urban R.G, Lane W.S, Gorga J.C, Stern L.J, Vignali D.A, Strominger J.L. 6389, August 1992, Nature, Vol. 358, pp. 764-8.
7. Emerging principles for the recognition of peptide antigens by MHC class I molecules. Matsumura M, Fremont D.H, Peterson P.A, Wilson I.A. 5072, August 1992, Science, Vol. 257, pp. 927-34.
8. Major Histocompatibility Complex (MHC) Class I and MHC Class II Proteins: Conformational Plasticity in Antigen Presentation. Wieczorek M, Abualrous E.T, Sticht J, Álvaro-Benito M, Stolzenberg S, Noé F, Freund C. March 2017, Frontiers in Immunology, Vol. 8, p. 292.
9. The MHC class I antigen presentation pathway: strategies for viral immune evasion. E.W, Hewitt. 2, October 2003, Immunology, Vol. 110, pp. 163-169.
10. Pathways of Antigen Processing. Blum J.S, Wearsh P.A, Cresswell P. January 2013, Annual Review of Immunology, Vol. 31, pp. 443-473.
11. Innate immunity: structure and function of TLRs. Delneste Y, Beauvillain C, Jeannin P. January 2007, Medical Science (Paris), Vol. 23, pp. 67-73.
12. The Structural Biology of Toll-like Receptors. Botos I, Segal D.M, Davies D.R. 4, April 2011, Structure, Vol. 19, pp. 447-459.
13. TLR10 is a Negative Regulator of Both MyD88-Dependent and Independent TLR Signaling. Jiang S, Li X, Hess N.J, Guan Y, Tapping R.I. 9, March 2017, Journal of Immunology, Vol. 196, pp. 3834–3841.
14. TLR10 is a B-cell Intrinsic Suppressor of Adaptive Immune Responses. Hess N.J, Jiang S, Li X, Guan Y, Tapping R.I. 2, January 2017, Journal of Immunology, Vol. 198, pp. 699-707.
15. Inflammasomes: far beyond inflammation. Henao-Mejia J, Elinav E, Strowig T, Flavell RA0. 4, March 2012, Nature Immunology, Vol. 13, pp. 321-4.
16. Inflammasomes: mechanism of action, role in disease, and therapeutics. Guo H, Callaway J.B, Ting J.P.Y. 7, July 2015, Nature Medicine, Vol. 21, pp. 677-687.
17. Syk kinase signalling couples to the Nlrp3 inflammasome for anti-fungal host defence. Gross O, Poeck H, Bscheider M, Dostert C, Hannesschläger N, Endres S, Hartmann G, Tardivel A, Schweighoffer E, Tybulewicz V, Mocsai A, Tschopp J, Ruland J. 7245, May 2009, Nature, Vol. 459, pp. 433-6.
18. Dectin-1 is an extracellular pathogen sensor for the induction and processing of IL-1β via a noncanonical caspase-8 inflammasome. Gringhuis S.I, Kaptein T.M, Wevers B.A, Theelen B, van der Vlist M, Boekhout T, Geijtenbeek T.B. 3, January 2012, Nature Immunology, Vol. 13, pp. 246-54.

TABLE OF CONTENTS

PerkinElmer®
For the Better

WORKS CITED

19. Inflammasome-Dependent Release of the Alarmin HMGB1 in Endotoxemia. Lamkanfi M, Sarkar A, Vande Walle L, Vitari A.c, Amer A.O, Wewers M.D, Tracey K.J, Kanneganti T.D, Dixit V.M. 7, October 2010, Journal of Immunology, Vol. 185, pp. 4385-4392.
20. Regulation of HMGB1 release by inflammasomes. Lu B, Wang H, Andersson U, Tracey K.J. [ed.] 167. 3, March 2013, Protein Cell, Vol. 4, p. 163.
21. FADD and caspase-8 mediate priming and activation of the canonical and noncanonical Nlrp3 inflammasomes. Gurung P, Anand P.K, Malireddi R.K, Vande Walle L, Van Opdenbosch N, Dillon C.P, Weinlich R, Green D.R, Lamkanfi M, Kanneganti T.D. 4, February 2014, Journal of Immunology, Vol. 192, pp. 1835-46.
22. The Macrophage. Verschoor C.P, Puchta A, Bowdish D.M.E. 2012, Methods in Molecular Biology, Vol. 844, pp. 139-56.
23. Exploring the full spectrum of macrophage activation. Mosser D.M, Edwards P.E. 12, August 2011, Nature Reviews of Immunology, Vol. 8, pp. 958-969.
24. Phenotypic and functional plasticity of cells of innate immunity: macrophages, mast cells and neutrophils. Galli S.J, Borregaard N, Wynn T.A. 11, October 2011, Nature Immunology, Vol. 12, pp. 1035-44.
25. Macrophage Polarization in Inflammatory Diseases. Liu Y.C, Zou X.B, Chai Y.F, Yao Y.M. 5, May 2014, Vol. 10, pp. 520-529.
26. Macrophage Extracellular Traps: A Scoping Review. Doster R.S, Rogers L.M, Gaddy J.A, Aronoff D.M. 1, 2018, Journal of Innate Immunology, Vol. 10, pp. 3-13.
27. Origin and Functions of Tissue Macrophages. Epelman S, Lavine K.J, Randolph G.J. 1, June 2014, Immunity, Vol. 41, pp. 21–35.
28. Physiological roles of macrophages. Gordon S, Martinez-Pomares L. 3, 2017, Pflügers Archiv: European Journal of Physiology, Vol. 469, pp. 365–374.
29. STEMCELLS Technologies Inc. Frequencies of Cell Types. 2017.
30. Living and dying for inflammation: neutrophils, eosinophils, basophils. Geering B, Stoeckle C, Conus S, Simon H.U. 8, August 2018, Vol. 34, pp. 398-409.
31. Evaluation of role of G-CSF in the production, survival, and release of neutrophils from bone marrow into circulation. Basu S, Hodgson G, Katz M, Dunn A.R. 3, August 2002, Blood, Vol. 100, pp. 854-61.
32. In vivo labeling with 2H2O reveals a human neutrophil lifespan of 5.4 days. Pillay J, den Braber I, Vrisekoop N, Kwast L.M, de Boer R.J, Borghans J.A, Tesselaar K, Koenderman L. 4, July 2010, Blood, Vol. 116, pp. 625-7.
33. Neutrophil granules: a library of innate immunity proteins. Borregaard N, Sørensen O.E, Theilgaard-Mönch K. 8, August 2007, Trends in Immunology, Vol. 28.
34. Neutrophil Extracellular Traps Kill Bacteria. Volker Brinkmann1, Ulrike Reichard1,Brinkmann V, Reichard U, Goosmann C, Fauler B, Uhlemann Y, Weiss D.S, Weinrauch Y, Zychlinsky A. 5663, March 2004, Science, Vol. 303, pp. 1532-1535.
35. Neutrophil extracellular traps in immunity and disease. V, Papayannopoulos. 2018, Nature Reviews of Immunoly, Vol. 18, pp. 134-147.
36. Polarization of Tumor-Associated Neutrophil (TAN) Phenotype by TGF-β: “N1” versus “N2” TAN. Fridlender Z.G, Sun J, Kim S, Kapoor V, Cheng G, Ling L, Worthen G.S, Albelda S.M. 3, September 2009, Cancer cell, Vol. 16, pp. 183-194.
37. IL-35 induces N2 phenotype of neutrophils to promote tumor growth. Jiu-Ming Zou, QinJ , Li Y.C, Wang Y, Li D, Shu Y, Luo C, Wang S.S, Chi G, Guo F, Zhang G.M, Feng Z.H. 20, April 2017, Oncotarget, Vol. 8, pp. 33501-33514.
38. Miltenyi Biotec. Granulocytes (human) - Human cell types - Human cells and organs - MACS Handbook - Resources - Miltenyi Biotec. miltenyibiotec.com. [Online] 2019. https://www.miltenyibiotec.com/US-en/resources/macs-handbook/human-cells-and-organs/human-cell-types/granulocytes-human.html.

TABLE OF CONTENTS

PerkinElmer®
For the Better

WORKS CITED

39. Eosinophils in vasculitis: characteristics and roles in pathogenesis. Khoury P, Grayson P.C, Klion A.D. 8, August 2014, Nature Reviews of Rheumatology, Vol. 10, pp. 474-83.
40. Accessory cell function of human eosinophils. HLA-DR-dependent, MHC-restricted antigen-presentation and IL-1 alpha expression. Weller P.F, Rand T.H, Barrett T, Elovic A, Wong D.T, Finberg R.W. 6, March 1993, Journal of Immunology, Vol. 150, pp. 2554-62.
41. The eosinophil. Rothenberg M.E, Hogan S.P. 2006, Annual Review of Immunolgy, Vol. 24, pp. 147-174.
42. Newly appreciated roles for basophils in allergy and protective immunity. Karasuyama H, Obata K, Wada T, Tsujimura Y, Mukai K. 9, September 2011, Allergy, Vol. 66, pp. 1133-41.
43. Basophils Produce IL-4 and Accumulate in Tissues after Infection with a Th2-inducing Parasite. Min B, Prout M, Hu-Li J, Zhu J, Jankovic D, Morgan E.S, Urban J.F, Dvorak A.M, Finkelman F.D, LeGros G, Paul W.E. 4, August 2004, Journal of Experimental Medicine, Vol. 200, pp. 507-517.
44. Beiträge zur Theorie und Praxis der histologischen Färbung. Ehrlich, P. Leipwig : s.n., 1878.
45. Decrease of mast cells in W/Wv mice and their increase by bone marrow transplantation. Kitamura Y, Go S, Hatanaka K. 2, August 1978, Blood, Vol. 52, pp. 447-52.
46. Origin of human mast cells: development from transplanted hematopoietic stem cells after allogeneic bone marrow transplantation. Födinger M, Fritsch G, Winkler K, Emminger W, Mitterbauer G, Gadner H, Valent P, Mannhalter C. 9, November 1994, Blood, Vol. 84, pp. 2954-9.
47. Identification and characterization of undifferentiated mast cells in mouse bone marrow. Jamur M.C, Grodzki A.C, Berenstein E.H, Hamawy M.M, Siraganian R.P, Oliver C. 11, June 2005, Blood, Vol. 105, pp. 4282-9.
48. Histamine in tissue mast cells. Riley J.F, West G.B. 4, June 1952, Journal of Physiology, Vol. 120, pp. 528–537.
49. Liberation of histamine and heparin by peptone from the isolated dog's liver. Rocha e Silva M, Scorggie A.E. 4, February 1947, Proceedings of the Society for Experimental Biology and Medicine, Vol. 62, pp. 141-6.
50. Mast Cell Function. A New Vision of an Old Cell. Marcelino da Silva E.Z, Jamur M.C, Oliver C. 10, 2014, Journal of Histochemistry and Cytochemistry, Vol. 62, pp. 698-738.
51. Mast cell repopulation of the peritoneal cavity: contribution of mast cell progenitors versus bone marrow derived committed mast cell precursors. Jamur M.C, Moreno A.N, Mello L.F, Souza Júnior D.A, Campos M.R, Pastor M.V, Grodzki A.C, Silva D.C, Oliver C. June 2010, BMC Immunology, Vol. 11, p. 32.
52. Mast cells: multifaceted immune cells with diverse roles in health and disease. Rao K.N, Brown M.A. November 2008, Annals of the New York Academy of Sciences, Vol. 1143, pp. 83-104.
53. Integrated signalling pathways for mast-cell activation. Gilfillan A.M, Tkaczyk C. 3, March 2006, Nature Review of Immunology, Vol. 6, pp. 218-30.
54. Revised map of the human progenitor hierarchy shows the origin of macrophages and dendritic cells in early lymphoid development. Doulatov S, Notta F, Eppert K, Nguyen L.T, Ohashi P.S, Dick J.E. 7, July 2010, Nature Immunology, Vol. 11, pp. 585-93.
55. Mature dendritic cells use endocytic receptors to capture and present antigens. Craig D. Platt, Jessica K. Ma, Cécile Chalouni, Melanie Ebersold, Hani Bou-Reslan, Richard A. D. Carano, Ira Mellman, and Lélia Delamarre. 9, March 2010, Proceedings of the National Academy of Sciences, Vol. 107, pp. 4287-4292.
56. Human dendritic cell subsets: an update. Collins M, Bigley V. 1, May 2018, Immunology, Vol. 154, pp. 3-20.
57. Human dendritic cell subsets. Collin M, McGovern N, Haniffa M. 1, September 2013, Immunology, Vol. 140, pp. 22-30.

TABLE OF CONTENTS

PerkinElmer®
For the Better

WORKS CITED

58. Plasmacytoid dendritic cells (PDC) are the major DC subset innately producing cytokines in human lymph nodes. Cox K, North M, Burke M, Singhal H, Renton S, Aqel N, Islam S, Knight S.C. 5, November 2005, Journal of Leucocyte Biology, Vol. 78, pp. 1142-52.

59. Plasmacytoid monocytes migrate to inflamed lymph nodes and produce large amounts of type I interferon. Cella M, Jarrossay D, Facchetti F, Alebardi O, Nakajima H, Lanzavecchia A, Colonna M. 8, August 1999, Nature Medicine, Vol. 5, pp. 219-23.

60. Human plasmacytoid dentritic cells elicit a Type I Interferon response by sensing DNA via the cGAS-STING signaling pathway. Bode C, Fox M, Tewary P, Steinhagen A, Ellerkmann R.K, Klinman D, Baumgarten G, Hornung V, Steinhagen F. 7, May 2016, European Journal of Immunology, Vol. 46, pp. 1615-21.

61. Plasmacytoid dendritic cells sense self-DNA coupled with antimicrobial peptide. Lande R, Gregorio J, Facchinetti V, Chatterjee B, Wang Y.H, Homey B, Cao W, Wang Y.H, Su B, Nestle F.O, Zal T, Mellman I, Schröder J.M, Liu Y.J, Gilliet M. 7162, October 2007, Nature, Vol. 449, pp. 564-9.

62. Plasmacytoid dendritic cells: sensing nucleic acids in viral infection and autoimmune diseases. Gilliet M, Cao W, Liu Y.J. 8, August 2008, Nature Review of Immunology, Vol. 8, pp. 594-606.

63. Self-RNA-antimicrobial peptide complexes activate human dendritic cells through TLR7 and TLR8. Ganguly D, Chamilos G, Lande R, Gregorio J, Meller S, Facchinetti V. 9, August 2009, Journal of Experimental Medicine, Vol. 206, pp. 1983-94.

64. Loss of TLR2, TLR4, and TLR5 on Langerhans cells abolishes bacterial recognition. Van Der Aar A.M, Sylva-Steenland R.M, Bos J.D, Kapsenberg M.L, de Jong E.C, Teunissen M.B. 4, February 2007, Journal of immunology, Vol. 178, pp. 1986-90.

65. CD1a, CD1b, and CD1c in immunity against mycobacteria. Van Rhijn I, Ly D, Moody D.B. 2013, Advances in Experimental Medicine and Biology, Vol. 783, pp. 181-97.

66. ranscriptional profiling of human dendritic cell populations and models unique profiles of in vitro dendritic cells and implications on functionality and applicability. Lundberg K, Albrekt A.S, Nelissen I, Santegoets S, de Gruijl T.D, Gibbs S, Lindstedt M. 1, January 2013, PLoS One, Vol. 8, p. e52875.

67. Miltenyi Biotec. Dendritic cells (human) - Human cell types - Human cells and organs - MACS Handbook - Resources - Miltenyi Biotec. miltenyibiotec.com. [Online] 2019. https://www.miltenyibiotec.com/US-en/resources/macs-handbook/human-cells-and-organs/human-cell-types/dendritic-cells-human.html.

68. Weizmann Institute of Science. CLEC9A Gene - GeneCards | CLC9A Protein | CLC9A Antibody. genecards.org. [Online] 2019. https://www.genecards.org/cgi-bin/carddisp.pl?gene=CLEC9A.

69. Biology of natural killer cells. Trinchieri, G. 1989, Advances in Immunology, Vol. 47, pp. 187-376.

70. Natural killer cells. In vitro and in vivo. C.S. Henney, D. Tracey, J.M. Durdik, G. Klimpel. 2, November 1978, The American Journal of Pathology, Vol. 93, pp. 459–46.

71. Constitutive Cytokine mRNAs Mark Natural Killer (NK) and NK T Cells Poised for Rapid Effector Function. D.B. Stetson, M. Mohrs, R.L. Reinhardt, J.L. Baron, Z.E. Wang, L. Gapin, M. Kronenberg, R.M. Locksley. 7, October 2003, Journal of Experimental Medicine, Vol. 198, pp. 1069–1076.

72. The biology of human natural killer-cell subsets. Cooper M.A, Fehniger T.A, Caligiuri M.A. 11, November 2001, Trends in Immunology, Vol. 22, pp. 633-40.

73. Activating receptors and coreceptors involved in human natural killer cell-mediated cytolysis. Moretta A, C. Bottino, M. Vitale, D. Pende, C. Cantoni, M. C. Mingari, R. Biassoni, L. Moretta. 2001, Annual Review of Immunology, Vol. 19, pp. 197-223.

74. The trafficking of natural killer cells. Grégoire C, Chasson L, Luci C, Tomasello E, Geissmann F, Vivier E, Walzer T. December 2007, Immunological Reviews, Vol. 220, pp. 169-82.

TABLE OF CONTENTS

PerkinElmer
For the Better

WORKS CITED

75. Human natural killer cells: their origin, receptors and function. Moretta L, Bottino C, Pende D, Mingari M.C, Biassoni R, Moretta A. 5, May 2002, European Journal of Immunology, Vol. 32, pp. 1205-11.
76. Human CD56bright NK Cells: An Update. Michel T, Poli A, Cuapio A, Briquemont B, Iserentant G, Ollert M, Zimmer J. 7, April 2016, Journal of Immunology, Vol. 196, pp. 2923-2931.
77. CD56bright cells differ in their KIR repertoire and cytotoxic features from CD56dim NK cells. Jacobs R, Hintzen G, Kemper A, Beul K, Kempf S, Behrens G, Sykora K.W, Schmidt R.E. 10, October 2001, European Journal of Immunology, Vol. 31, pp. 3121-7.
78. Unique subpopulations of CD56+ NK and NK-T peripheral blood lymphocytes identified by chemokine receptor expression repertoire. Campbell J. J, S. Qin, D. Unutmaz, D. Soler, K. E. Murphy, M. R. Hodge, L. Wu, E. C. Butcher. 11, June 2001, Journal of Immunology, Vol. 166, pp. 6477-82.
79. Comparative studies of human FcRIII-positive and negative natural killer cells. Nagler A, Lanier L.L, Cwirla S, Phillips J.H. 10, November 1989, Journal of Immunology, Vol. 143, pp. 3183-91.
80. Human natural killer cells: a unique innate immunoregulatory role for the CD56bright subset. Cooper M.A, Fehniger T.A, Turner S.C, Chen K.S, Ghaheri B.A, Ghayur T, Carson W.E, Caligiuri M.A. 10, May 2001, Blood, Vol. 97, pp. 3146-51.
81. Selective rejection of H-2-deficient lymphoma variants suggests alternative immune defence strategy. Kärre K, Ljunggren H.G, Piontek G, Kiessling R. 6055, February 1986, Nature, Vol. 319, pp. 675-8.
82. In search of the 'missing self': MHC molecules and NK cell recognition. Ljunggren H.G, Kärre K. 17, July 1990, Immunology Today, Vol. 11, pp. 237-44.
83. Functions of natural killer cells. Vivier E, Tomasello E, Baratin M, Walzer T, Ugolini S. 5, May 2008, Nature Immunology, Vol. 9, pp. 503-10.
84. Arousal and inhibition of human NK cells. Lanier L.L, Corliss B, Phillips J.H. February 1997, Immunological Reviews, Vol. 155, pp. 145-54.
85. Ectopic expression of retinoic acid early inducible-1 gene (RAE-1) permits natural killer cell-mediated rejection of a MHC class I-bearing tumor in vivo. Cerwenka A, Baron J.L, Lanier L.L. 20, September 2001, Proceedings of the National Academy of Sciences, Vol. 98, pp. 11521-6.
86. NK cell recognition. Lanier, L.L. 2005, Annual Review of Immunology, Vol. 23, pp. 225-74.
87. Natural-killer cells and dendritic cells: «l'union fait la force». Walzer T, Dalod M, Robbins S.H, Zitvogel L, Vivier E. 7, October 2005, Blood, Vol. 106, pp. 2252-8.
88. Dendritic Cells Prime Natural Killer Cells by trans-Presenting Interleukin 15. Lucas M, Schachterle W, Oberle K, Aichele P, Diefenbach A. 4, April 2007, Immunity, Vol. 26, pp. 503-17.
89. Close encounters of different kinds: dendritic cells and NK cells take centre stage. Degli-Esposti M.A, Smyth M.J. 2, February 2005, Nature Reviews of Immunology, Vol. 5, pp. 112-24.
90. T-bet and GATA3 orchestrate Th1 and Th2 differentiation through lineage-specific targeting of distal regulatory elements. Kanhere A, Hertweck A, Bhatia U, Gökmen M.R, Perucha E, Jackson I, Lord G.M, Jenner R.G. 2012, Nature Communications, Vol. 3, p. 1268.
91. RORC2: the master of human Th17 cell programming. Unutmaz, D. 6, June 2009, European Journal of Immunology, Vol. 39, pp. 1452-5.
92. RORγt and RORα signature genes in human Th17 cells. Castro G, Liu X, Ngo K, De Leon-Tabaldo A, Zhao S, Luna-Roman R, Yu J, Cao T, Kuhn R, Wilkinson P, Herman K, Nelen M.I, Blevitt J, Xue X, Fourie A, Fung-Leung W.P. 8, August 2017, PLos One, Vol. 12, p. e0181868.

TABLE OF CONTENTS

PerkinElmer® *For the Better*

WORKS CITED

93. When worlds collide: Th17 and Treg cells in cancer and autoimmunity. Knochelmann H.M, Dwyer C.J, Bailey S.R, Amaya S.M, Elston D.M, Mazza-McCrann J.M, Paulos C.M. March 2018, Cellular and Molecular Biology.
94. Differentiation, regulation and function of Th9 cells. Jia l, Wu C. 1, 2014, Advances in Experimental Medicine and Biology, Vol. 841, pp. 181-207.
95. The biology and functions of Th22 cells. Jia L, Wu C. 2014, Advances in Experimental Medicine and Biology, Vol. 841, pp. 209-30.
96. T follicular helper cell differentiation, function, and roles in disease. Crotty, S. 4, October 2014, Immunity, Vol. 41, pp. 529–542.
97. Naturally occurring regulatory T cells: markers, mechanisms, and manipulation. Schmetterer K.G, Neunkirchner A, Pickl W.F. 6, June 2012, FASEB Journal, Vol. 26, pp. 2253-76.
98. Molecular Mechanisms of Treg-Mediated T Cell Suppression. Schmidt A, Oberle N, Krammer P.H. March 2012, Frontiers in Immunology, Vol. 3, p. 51.
99. An essential role for the IL-2 receptor in Treg cell function. Chinen T, Kannan A.K, Levine A, Fan X, Klein U, Zheng Y, Gasteiger G, Feng Y, Fontenot J.D, Rudensky A.Y. 11, November 2016, Nature Immunology, Vol. 17, pp. 1322-1333.
100. CD127 expression inversely correlates with FoxP3 and suppressive function of human CD4+ T reg cells. Liu W, Putnam A.L, Xu-Yu Z, Szot G.L, Lee M.R, Zhu S, Gottlieb P.A, Kapranov P, Gingeras T.R, Fazekas de St Groth B, Clayberger C, Soper D.M, Ziegler S.F, Bluestone J.A. 7, July 2006, The Journal of Experimental Medicine, Vol. 203, pp. 1701-11.
101. The regulation of immune tolerance by FOXP3. Lu L, Barbi J, Pan F. 11, November 2017, Nature Review of Immunology, Vol. 17, pp. 703-717.
102. Forkhead box P3 regulates ARHGAP15 expression and affects migration of glioma cells through the Rac1 signaling pathway. Zhen Sun, Biao Zhang, Chen Wang, TAo Fu, Lianling Li, Qiaoli Wu, Ying Cai, Jinhuan Wang. 1, January 2017, Cancer Science, Vol. 108, pp. 61-72.
103. Immunologic self-tolerance maintained by CD25(+)CD4(+) regulatory T cells constitutively expressing cytotoxic T lymphocyte-associated antigen 4. Takahashi T, Tagami T, Yamazaki S, Uede T, Shimizu J, Sakaguchi N, Mak T.W, Sakaguchi S. 2, July 2000, The Journal of Experimental Medicine, Vol. 192, pp. 303-10.
104. CD4(+)CD25(+) immunoregulatory T cells: gene expression analysis reveals a functional role for the glucocorticoid-induced TNF receptor. McHugh R.S, Whitters M.J, Piccirillo C.A, Young D.A, Shevach E.M, Collins M, Byrne M.C. 2, February 2002, Immunity, Vol. 16, pp. 311-23.
105. A CD4+ T-cell subset inhibits antigen-specific T-cell responses and prevents colitis. Groux H, O'Garra A, Bigler M, Rouleau M, Antonenko S, de Vries J.E, Roncarolo M.G. 6652, October 1997, Nature, Vol. 389, pp. 737-42.
106. Generation and Function of Induced Regulatory T Cells. Schmitt E.G, Williams C.B. June 2013, Frontiers in Immunology, Vol. 4, p. 152.
107. TGF-beta 1 plays an important role in the mechanism of CD4+CD25+ regulatory T cell activity in both humans and mice. Nakamura K, Kitani A, Fuss I, Pedersen A, Harada N, Nawata H, Strober W. 2, January 2004, Journal of Immunology, Vol. 172, pp. 834-42.
108. Two functional subsets of FOXP3+ regulatory T cells in human thymus and periphery. Ito T, Hanabuchi S, Wang Y.H, Park W.R, Arima K, Bover L, Qin F.X, Gilliet M, Liu Y.J. 6, June 2008, Immunity, Vol. 28, pp. 870-80.
109. Adenosine generation catalyzed by CD39 and CD73 expressed on regulatory T cells mediates immune suppression. Deaglio S, Dwyer K.M, Gao W, Friedman D, Usheva A, Erat A, Chen J.F, Enjyoji K, Linden J, Oukka M, Kuchroo V.K, Strom T.B, Robson S.C. 6, June 2007, Journal of Experimental Medicine, Vol. 204, pp. 1257-65.
110. Nucleotide- and nucleoside-converting ectoenzymes: Important modulators of purinergic signalling cascade. G.G, Yegutkin. 5, May 2008, Biochimica et Biophysica Acta, Vol. 1793, pp. 673-94.

TABLE OF CONTENTS

PerkinElmer
For the Better

WORKS CITED

111. CTLA-4 control over Foxp3+ regulatory T cell function. Wing K, Onishi Y, Prieto-Martin P, Yamaguchi T, Miyara M, Fehervari Z, Nomura T, Sakaguchi S. 5899, October 2008, Science, Vol. 322, pp. 271-5.

112. The B7 family of immune-regulatory ligands. Collins M, Ling V, Carreno B.M. 6, May 2005, Genome Biology, Vol. 6, p. 223.

113. CTLA4-Ig immunosuppressive activity at the level of dendritic cell/T cell crosstalk. Mayer E, Hölzl M, Ahmadi S, Dillinger B, Pilat N, Fuchs D, Wekerle T, Heitger A. 3, March 2013, International Immunopharmacology, Vol. 15, pp. 638–645.

114. Trans-endocytosis of CD80 and CD86: a molecular basis for the cell extrinsic function of CTLA-4. Qureshi O.S, Zheng Y, Nakamura K, Attridge K, Manzotti C, Schmidt E.M, Baker J, Jeffery L.E, Kaur S, Briggs Z, Hou T.Z, Futter C.E, Anderson G, Walker L.S.K, Sansom D.M. 6029, April 2011, Science, Vol. 332, pp. 600-603.

115. Human T regulatory cells can use the perforin pathway to cause autologous target cell death. Grossman W.J, Verbsky J.W, Barchet W, Colonna M, Atkinson J.P, Ley T.J. 4, October 2004, Immunity, Vol. 21, pp. 589-601.

116. Two modes of immune suppression by Foxp3(+) regulatory T cells under inflammatory or non-inflammatory conditions. amaguchi T, Wing J.B, Sakaguchi S. 6, December 2011, Seminars in Imunology, Vol. 23, pp. 424-30.

117. CD4+CD25+Foxp3+ regulatory T cells induce cytokine deprivation-mediated apoptosis of effector CD4+ T cells. Pandiyan P, Zheng L, Ishihara S, Reed J, Lenardo M.J. 12, December 2007, Nature Immunology, Vol. 8, pp. 1353-62.

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