Immunology and Pathology

Notes from lectures

Bioinformatics

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Introduction to cells, organs and function of immune system

Role and properties

The immune system recognizes microorganisms that are potentially dangerous, stops their spread, and eradicates it. Tumors are also recognized by the immune system, these transformed cells are recognized and destroyed, limiting cancer growth. Lastly, the immune system can also attack non infectious forign substances, such is the case of allergies in which inflammation is induced. We can also train the immune system from transplants in gene therapy, or vaccines.

Cytotoxic lymphocytes are the immune response element that kills everything it attacks, even healthy cells. The other immune response is an antibody-mediated one, which antibodies are secreted. There are two phases to this antibody response, the first are B-Cells, which make IgG, once the antibody level again decreases, a second infection will invoke memory B-Cells which are able to act faster and the memory B-cell level will remain high longer.

Reverse Vaccinology is the streamlining of vaccine development through bioinformatics tools which allow structural prediction of vaccine targets and mechanisms. This allows vaccines to be developed faster, and even cracking the vaccine puzzle is hard to solve for organisms.

The three important properties of the immune system are versatility, recognition of self from non-self, and the diverse immune responses. When any of these elements are compromised, the integrity of the system breaks down. Disorders in recognition cause autoimmune symptoms, when the system cannot recognize self and damages own cells, or can't recognize a pathogen

Innate Vs Adaptive

The immune system can be broken into innate immunity, or adaptive immunity. Innate provides early defense to microbes, exploiting general defense mechanisms regardless of the pathogen, and its main function is to limit the infection. Innate responses are fast because they contain the effector function unlike adaptive which needs to recruit other cells. Adaptive immunity, or adaptive immunity provides later response, but is pathogen specific and ultimately is responsible for eradicating the infection and providing protection. The adaptive immune system has two arms, the antibody response which neutralizes pathogens and prevents infections, while the T-Cell mediated arm kills infected cells, and produces antiviral cytokines.

Forign substances or damaged self are recognized by the immune system leading to inflammation. Inflammatory cells accumulate and destroy the stimulus, followed by the removal of the forign molecule and the inflammatory cells by phagocytosis, and finally tissue repair. Chronic inflammation causes most known diseases, and results in the inappropriate activation of the immune system (Autoimmune disorder) or through the failure to inactivate the inflammation stimuli.

Barriers

The first line of defense is the epithelium. Epithelial surfaces are external such as the skin, or

internal such as the mucosa (respiratory, urogenital, gastrointestinal). In the gastrointestinal tract good bacteria compete with bad bacteria defending us more. Bacteria and viruses generally are intercellular while fungi and parasites are extracellular. Damages from microbes can be exotoxins, endotoxins, killing of the host cell, or inappropriate immune response. Exotoxins are the most dangerous, as they can kill hundreds of cells instantly. Endotoxins and some viruses destroy the cell they inhabit, while many microbes can induce autoimmune response which can cause chronic inflammation.

Lymphatic System: organs and tissues

Lymphocytes patrol the body through blood and lymphatic vessels connecting organs and tissues. Naive lymphocytes have high activation potential based on their activation catalyst, meaning the antigen can be collected in secondary lymphoid tissues, and activated in optimal conditions in these tissues.

Therefore the phases of the adaptive immune response is the exit of the naive lymphocytes from primary lymphoid organs from mature T or B cells, followed by the activation of the lymphocytes into effector lymphocytes through differentiation. These differentiated effectors can be antibody secreting plasma cells, (B cells) or Helper T or cytotoxic T (T cells). Lastly, the memory lymphocytes are activated at the time of effector activation, but remain in the circulation system to detect future infections by the same antigen. After antigen recognition, lymphocytes activate and proliferate before converting to effectors allowing them to accumulate in number for more efficient response.

Primary Lymphoid organs

The thymus is the site of T cell maturation, and is lobules regions separated by connective tissues. The outermost part (outer cortex) is populated by thymocytes and epithelial cells specialized in antigen presentation called cortical thymic epithelial cells (cTEC). The medullary area contains less thymocytes and cells expressing MHC-I and II. These thymocytes are immature T cells.

Bone marrow is responsible for both the creation of T cell precursors and the production and maturation of B cells. Bone marrow contains hematopoietic and non hematopoietic cells, containing a porous bone area and a trabecular area inside (bone fragments). The endosteum separates the bone and the bone marrow, and contains cells differentiated into osteoblasts. From the bone marrow, B cells immediately join the circulatory system and travel to secondary lymphoid organs in search of pathogens. T cells, on the other hand, travel from the bone marrow to the thymus, where they develop further and mature. Mature T cells then join B cells in search of pathogens. The other 95% of T cells begin a process of apoptosis, a form of programmed cell death.

Secondary lymphoid organs

Peripheral lymphoid organs are sites of lymphocyte differentiation and depend on exogenous antigens. These are lymph nodes, responsible for introducing antigens from tissues to lymphatic vessels. The spleen responds to blood antigens, while MALT (mucosa associated lymphoid tissues control the entry of microbes from organisms that can overcome the epithelial barrier.

Lymph (interstitial fluid) comes from blood vessel leakage, most of which is recaptured locally, but 10% is left in the extravascular space. From here, the fluid is drained through the unidirectional

lymphatic vessels, and deposited into the thoracic duct, and back into the bloodstream through the subclavian vein (near the heart).

Lymph nodes are located on lymphatic vessels, allowing the lymph response to be localized such as the case of absorption from the lymph nodes in the legs. The substance of a lymph node consists of lymphoid follicles in an outer portion called the cortex. The inner portion of the node is called the medulla. The arteries and veins supplying the lymph node with blood enter and exit through the hilum. The region of the lymph node called the paracortex immediately surrounds the medulla. Unlike the cortex, which has mostly immature T cells, or thymocytes, the paracortex has a mixture of immature and mature T cells. Lymphocytes enter the lymph nodes through specialised high endothelial venules found in the paracortex. A lymph follicle is a dense collection of lymphocytes, the number, size, and configuration of which change in accordance with the functional state of the lymph node. For example, the follicles expand significantly when encountering a foreign antigen. The selection of B cells occurs in the germinal centre of the lymph nodes, meaning the antigen has been recognized and there is rapid proliferation. Secondary lymphoid tissue provides the environment for the foreign or altered native molecules (antigens) to interact with the lymphocytes.

The B and T cells in the follicles are tightly separated therefore coming in close contact with cells of the same type. This segregation is achieved through chemokine activity, mice lacking chemokines also lack this segregation. Chemokines can be broken into four basic families, CXC (cysteine other cysteine) CC, C, CX3C. These chemokine receptors are G-protein coupled receptors (seven membrane crosses) and signal through allosteric conformational change of the chemokine receptor inducing the released of the B and Gamma subunits from the A subunit by the phosphorylation of GDP to GTP. This A-Gamma subunit is able to interact with cell receptors inducing a chemotaxis response, or the promotion of adhesion of a cell to a specific surface. This adhesion promotes entrance of a cell from the blood into a tissue, first by receptor mediated adhesion. One of these adhesion signals is the collection of B cells into follicles via the CXCL-13 (produced by bollical dendritic cells binding CXCR5 receptor expressed on naive B cells.

Spleen

The spleen is composed of an external capsule of connective tissue. The internal structure is divided into red pulp containing venous vessels and macrophages as well as red blood cells, and the white pulp. The main functions of the spleen are: to produce immune cells to fight antigens, to remove particulate matter and aged blood cells, mainly red blood cells and to produce blood cells during fetal life.

The spleen synthesizes antibodies in its white pulp and removes antibody-coated bacteria and antibody-coated blood cells by way of blood and lymph node circulation. These monocytes, upon moving to injured tissue (such as the heart), turn into dendritic cells and macrophages while promoting tissue healing. The spleen is a center of activity of the mononuclear phagocyte system and can be considered analogous to a large lymph node, as its absence causes a predisposition to certain infections.

Mucosa-associated lymphoid tissue

MALT consists of lymphocytes located in the epithelial layer, and in the gut there are the PEYER patches which limit the entry into epithelial tissues. MALT is a diffuse system of small concentrations of lymphoid tissue found in various submucosal membrane sites of the body, such as the gastrointestinal tract or skin. MALT is populated by lymphocytes such as T cells and B cells, as well as plasma cells and macrophages, each of which is well situated to encounter antigens passing through the mucosal epithelium. In the case of intestinal MALT, M cells are also present, which sample antigen from the lumen and deliver it to the lymphoid tissue. MALT constitutes about 50% of the lymphoid tissue in the human body.

Lymphanoids are spread out in many different tissues, mainly in the form of MALT and lymph nodes. The Spleen instead is a single organ which is densely packed with these lymphocyte cells, but in any tissue exposed to environmental microbes (Skin, intestine, lungs) contain more dispersed lymphoid cell tissues.

Adaptive System overview

The adaptive immune system is slower, and more specific than the innate immune system. Antigens are able to be recognized by T and B lymphocyte cells which are complementary to some element of the pathogens cell. These lymphocyte cells are able to develop with such specificity through somatic recombination forming specific receptors during lymphocyte maturation. This maturation is through random events, allowing the B and T cells to adapt to new pathogens using rudimentary protein domains to form novel receptors -from recombination and mutations- which are antigen specific. This puts selective pressure on the T and B cells which are able to correctly form receptors which complement.

Clonal Selection

The clonal selection hypothesis tells us that lymphocytes formed through random recombination which are able to bind to the antigen are selected to proliferate (Clonal Selection) , while those which cannot recognize the antigens are negatively selected and stop proliferating. These unactivated B and T cells are in a naive state in which they are not rapidly proliferating, but are ready in case the complementary antigen appears.

T and B cell receptors are specific, and can recognize around 1 million antigens compared to 1000 molecular patterns in the innate. Only two types of receptors exist in adaptive, either Ig (immunoglobulin) or TCR. While innate cells come from germline genes, adaptive cells come from somatic recombination.

Innate immunity ***

The major components of the innate system are cells (Phagocytes, Natural Killers, granulocytes) and molecules (complements, peptides, enzymes, cytokines). Innate immunity is characterized by speed, constantutivness, effector function, and the generalization of the response.

Epithelial barriers are the edge of the immune system, either in the gastrointestinal and respiratory tract, or the skin. Lysozymes in the gastrointestinal tract cleave the transpeptidation of the

peptidoglycan in bacteria to disintegrate the cell wall. Mucus, and stomach acids are also secreted by gastrointestinal cells to neutralize and destroy microbes.

Innate Immune cells

Most immune cells are white blood cells (leukocytes), which patrol the system and remove infections from tissues. Innate immune cells are:

- **Neutrophils**- Neutrophils are the granulocytic leukocytes They are the first cells that act at the site of tissue damage to eliminate pathogens especially bacteria by phagocytosis. A type of cell located in the bloodstream, quickly ingesting and destroying microorganisms. Neutrophils rise in number in the bloodstream through illness and are primarily responsible for the elevated amount of white blood cells associated with some infections.
- **Eosinophils**- Eosinophils are also granulocytic leukocytes. They release the contents of their granules to kill pathogens. They produce a variety of growth factors and cytokines and help other immune cells.
- **Basophils**- Basophils are the largest granulocytic leukocytes. They have histamine-rich granules and are involved in inflammatory responses. They help in the secretion of cytokines involved in the maturation of T-helper cells.
- **Monocytes**- A type of phagocytic cell located in the bloodstream that, when moving to tissues, transforms into a macrophage. Monocytes are the largest white blood cells that produce Macrophages & Dendritic cells.
- **Macrophages** engulf and process cell debris, pathogens, and cancer cells. They are involved in wound healing, tissue regeneration, and pro-inflammatory activities as well as activation of bactericidal mechanisms. Found in tissue and present antigen to T cells in the tissues.
- **Dendritic cells** are present in barrier tissues nes. They present antigens to B and T cells and also secretes cytokines. Sensitive cells introduce antigen to T cells outside tissue.
- **Mast cells**- are granulocytes that secrete heparin, histamine, and other factors. These secretions are important in activating blood vessels so they can be opened. They help in wound healing, angiogenesis, and elimination of parasites.
- **Natural killer (NK) cells** (May develop from both Myeloid and Lymphoid progenitor). NK cells are produced from the bone marrow and are found in the bloodstream and tissues in reasonably low quantities. They are cytotoxic cells that have small granules with perforins and granzymes and destroy infected cells and cancer cells rapidly.

All blood cells are derived from the common precursor found in bone marrow called hematopoietic stem cells through the process of hematopoiesis. Hematopoietic stem cells divide into one precursor cell and one new stem cell, these cells are not differentiated.

Recognition

Microbes are recognized by pattern recognition receptors such as toll, mannose, N-formylmethionyl receptor, and scavenger receptors. These receptors are specific for different classes of pathogens, thus they can recognize many different pathogens based on molecular pattern recognition. dsRNA is also a strong indicator for receptors of a pathogen, as well as lipopolysaccharide. Another possible recognition target is the bacterial cell wall, especially the peptidoglycan, but also flagellin,

porin, mannose, pilin, or lipoteichoic and teichoic acids. These kind of receptors are pathogen associated molecular pattern (PAMP) binding, but we also have damage-associated molecular patterns (DAMP) which recognizes stress molecules or nuclear proteins (indicating DNA damage)

Functional classes of receptors can be divided into three classes:

1. Endocytic pattern recognition: Engulfment of pathogens to clear excessive toxic material. Bound materials are engulfed into phagosomes and fuze with lysosomes
    a. C-Type lectin receptor: C receptors are able to recognize a variety of glycans such as mannose receptor and B-Glucan receptor.
    b. Scavenger receptors: Modify LDL, and bind microbes by LPS, PG ect. And in macrophages assist in apoptosis of host cells
2. Chemotactic pattern recognition: Migration to infection site
3. Signal Pattern recognition: Production of effectors of immune response
    a. TLR (Toll-Like) found in plasma membrane, or endosome membrane, the ones in the PM recognize components of the bacterial surface, while the vesical TLR recognize microbial molecules released following phagocytosis/endocytosis. TLRs also recognize proteins involved in cellular stress response. TLR always work as dimers, homodimers or hetero, and are found in almost all inate immune cells. The recognition domain is composed of a leucine rich motif, and the main outcome is gene activation.
        i. LPS from gram- bacteria are recognized by CD14, which is associated with TLR-4 who transduces the signal and promotes the transcription of cytokines as well as the induction of the adaptive response through the activation of dendritic cells
    b. NLR (Node Like) found in the cytosol of phagocytes, and epithelial cells
    c. RLR (Rib-like) found in cytosol of phagocytes and other cells
    d. PRR (Pathogen Recognition Receptors) circulate the system and through complement system bind the microbe to induce an immune response

Response

Once recognized, the pathogens are phagocytized, killed, or recruit other cells to help. This recruit is through a molecular messenger called a cytokine, which activated mobilization of cells. Chemokines are cytokines with chemotactic activity. Cytokines are low molecular weight glycoproteins which bind to the receptor and mediate the length and intensity of the immune response. Cytokines mainly work locally through autocrine action (same cell) or paracrine action (nearby cell) or rarely through endocrine action (circulation).

Bacteria can enter into the system when there is a break in the barrier such as a cut. In this case, vasoactive and chemotactic factors are released and promote the entry of phagocytic cells from the capillaries into the tissue damage site. The entry of microbes or damage of the tissue are perceived in the same way and response is inflammation. Inflammation protects from exogenous microbes and also repairs the damage caused to the tissue. Inflammation response is very heterogeneous, so examining the inflammation can tell us more information about the response which can be acute or chronic, and local or systemic.

Antigen Capture and Presentation to Lymphocytes: ***

T and B recognition

T and B recognize structures called antigens, which can be proteins , polysaccharides, and lipids. T cells can only recognize proteins, while B can recognize any of the motifs. Proteins are the best antigen, because they can be recognized by all lymphocyte cells. T and B cells which recognize self are eliminated in development, failure to eliminate them causes allergies. The epitope of Antigen determinant is the specific region of the antigen that interacts with the recognition site of antigen receptors.

T and B lymphocytes are both responsible for antigen recognition in the adaptive immune response. This response is activated when pathogens overcome the innate immune response. Adaptive immunity can be divided into humoral immunity or antibody immunity and cell mediated immunity. Humoral immunity comes from B lymphocytes which secrete antibodies able to block infections and eliminate extracellular microbes. Cell-mediated immunity instead is orchestrated by helper-T and cytotoxic-T cells which activate macrophages or kill infected cells respectively, both activated when the infection is already inside the cells.

T and B cells recognize their antigens differently. B uses BCR (B cell receptor) and T uses TCR. The BCR is highly variable, composed of two antigen binding sites in a y shape, the arms rotate and interact with the antigen. TCR has two chains, the a and b with the antigen binding site at the top which is highly variable as well. These differences mean that BCR recognizes proteins in its native state, while TCR interacts with denatured and linear antigens. This also means BCR does not need other proteins, while TCR requires the proteins to be processed before it can bind, and also requires another accessory cell called the MHC.

MHC

Major Histocompatibility Complexes or MHC come in two classes, class I is expressed in all cells with a nucleus, while class II is only present in highly specialized cells for antigen presentation such as macrophages. An antigen in the presence of TCR does not produce a T cell response, but when MHC is present the T cells will be activated. The epitope for TCR is usually buried inside the folded protein, so the protein must be degraded and presented by accessory cells, then recognized first by the MHC followed by the TCR binding to the complex.

MHC class-I present mainly self cells, until the system is infected with the pathogen. This was discovered through organ donors who did not recognize the transplant as self and attacked it. MHC-Restriction is also seen in transplants when the transplanted T cells from an organism with viral resistance to an organism with the MHC receptor cannot respond to the virus. MHC and TCR link at the a and b chains, as well as the T cell Contact residue by the anchor residue of the MHC. This means all three interactions must be specific and satisfied in order for the complex to be active, called the trimolecular interaction. For a self peptide, the T-cell receptor won't be triggered, and the complex will remain inactive, which is the important point of MHC restriction, while MHC does not contain self vs non-self recognition, the TCR does.

MHC genes are highly polymorphic present in different varieties, as well as being polygenic, meaning we receive many MHC receptors from each parent. Each chromosome contains three class-I MHC (6 per cell), while we can have many class-II, even more because they dimerize in different combinations. We need many different MHC genes in order to provide resistance to microbes which mutate to evade one MHC recognition. We tend to consider people with different HLA proteins more attractive than those with the same.

Antigen Presenting Cells such as dendritic cells, macrophages and B-Cells all present class-II MHC receptors and present these antigens to the T cells.

B-Cell Antibody structure

BCR are composed of a heavy chain and a light chain, and are formed in bone marrow. The membrane bound antibody is composed of a conserved chain attached to a variable portion, forming two functionally different domains, the variable domain binds with the antigen, while the conserved domain allows the activation of IgA and IgB for signalling inside the cell. Antibodies are also called immunoglobulins, abbreviated to Ig. These antibodies can be secreted as well in the case of the humorous adaptive response, or membrane bound such as in B cell maturation. Antigen epitopes can be different shapes in order to interact with variable surfaces on the target. BCR and antibodies contain immunoglobulin domains made by beta sheets allowing formation of a planar surface which facilitates interaction. The conserved domain of membrane bound BCR contains 3 immunoglobulin domains per chain, and the variable region has two per chain, while in the case of secreted antigens we see only two in conserved and two in variable. Each immunoglobulin domain is made of three antiparallel beta sheets, and interestingly the most variable regions of the antibody are found in the amino acids connecting these B-sheets. Secreted antigens are pentamers instead of dimers. When there is one interaction we have low avidity (interaction strength), two for receptor means very high avidity, while secreted antibodies binding all sites are very strong avidity.

Another region of the BCR is the hinge, found between the conserved and the variable regions. The hinge region is highly conserved among antibodies and is responsible for orienting the Ab binding sites so that two antigens can dimerize. Papain enzymes cleave antigens in three parts, each variable chain is cleaved into a Fab (Fragment, antigen binding) while Fc (fragment, crystallizable) is left. Antibodies are divided into distinct classes called isotypes, and subclasses based on their heavy chain C regions.

- IgG is responsible for diverse functions in the blood including complement activity, cell-mediated cytotoxicity, and has a long half life.
- IgA instead is found mainly in mucosa in the international tract, and plays a role in blocking the entry of microbes by blocking parasites.
- IgM works as a mauve cell antigen receptor, and works in complement activation.
- IgE has a short halflife, and works as a defense against helminth parasites.
- IgD is used only in receptor function

T-Cell Antibody Structure

As we know, B cells recognize the antibody in its native conformation, while TCR needs the peptide

to be processed, as well as requiring the MHC in order to bind the epitope. Both BCR and TCR contain variable portions, and need chains associated with the receptor to perform function, while TCR has no effector role and BCR does. TCR interacts through the trivalent interaction between TCR, antigen, and MHC.

Antigens are loaded in MHC-II dimers between the b and a chain, while in class-I it is loaded between the 3rd and 2nd chain of the a chain. Both I and II contain an immunoglobulin chain which stabilizes the chains and a peptide binding cleft at the top. The structure of the binding groove is what specifies the length of peptides which the MHC can bind, class II has a open cleff which accommodates longer peptide binding, while class I clefts are formed by a single chain, and is closer to a pocket that is limited in the length of peptides which can fit, between 8-11 amino acids. Binding the peptide is required to stabilize the heterodimer, therefor without it MHC cannot bind the cell membrane, and MHC has no discrimination between self and forign peptides. Very few MHC-II complexes contain the same peptide on the cell surface, but this is very rare. Each MHC molecule can also bind many peptides of different lengths due to the relatively few anchor residue pockets compared to total peptide length. The anchor sites that connect MHC to TCR are the most polymorphic sites and give MHC the specificity for a TCR.

Professional Antigen presenting cells

MHC-II expressing cells are called professional antigen expressing cells, and are B-cells, macrophages, dendritic cells and some epithelial cells in the thymus. Cytokines upregulate MHC expression, type I interferons (INF a/b) and type II by interferon type II (IFN gamma). Professional APC have high expression of both classes of MHC and also co-stimulatory molecules which activate CD4+ and CD8+ T cells. This secondary signal is required for T cells to know the presented antigen is not a self molecule, and occurs only in secondary lymphoid organs. The immune system avoids the presentation of antigens to organs other than secondary lymphoid organs in order to prevent an autoimmune response.

Once activated, dendritic cells activate naive T-cells promoting differentiation and effector activation. Macrophages which have phagocytized a microbe increase MHC II expression and activate effector T cells. The activation from macrophages is linked to the expression of MHC II, and therefore the amount of pathogen present. Finally, B cells instead of macrophages require a secondary signal from an effector T cell, which promotes their transformation into antigen producing B cells.

Dendritic cells originate in bone marrow and are present in body surfaces, peripheral organs, and T cell dependent areas of lymphoid organs. These dendritic cells mature in response to microbes and stimuli such as cytokines. Tissue resident dendritic cells transport and present the antigen using MHC I or II.

Antigens are processed and presented differently for MHC I and II. For class I, proteases digest the protein, followed by the small peptides binding to receptors in the endoplasmic membrane, where they can be transported to T cells via exocytic vesicles. In class II, the proteins are packaged into phagosomes first for degradation, followed by proteases, and absorption into ER as class I.

Lymphoid maturation

B and T lymphocytes come from different generative lymphoid organs, however the stem cell precursors all come from bone marrow. T comes from the thymus organ, while B comes from bone marrow. The cells exiting from these generative organs are termed naive, but they are in fact mature, simply not activated as an effector yet. T and B enter organs and tissues through the blood, and recirculate out of the secondary tissues back into the blood. The hematopoietic system maintains the equilibrium in the blood circulation of mature cells from various processes not limited to the immune system.

Each cell has a CD (Cluster designation) which is used to identify monoclonal antibodies, and numerated in order to distinguish one cell from another, as well as one activation state from another. For example, T helper and cytotoxic T lymphocytes appear to be the same in microscopy, yet we know T helper has CD4 and cytotoxic T has CD8, so we can distinguish them.

Lymphocytic Activation

Both lymphocytes are activated in peripheral tissues (secondary lymphoid organs) by the migration of dendritic cells to the lymph nodes (via lymph vessels) with the captured antigens, producing inflammation. These dendritic cells in the lymph nodes wait for naive T and B cells to induce activating, entering circulation and producing the specific immune response. Here, the cytotoxic T (CTT) cells activate macrophages, while antibody secreting B cells produce complementary antibodies.

Lymphocyte Homing

Homing cal be constitutive or inducible, and carries the lymphocytes to the microbe invasion. Immune surveillance is the process of the lymph node patrolling the system called constitutive, or in tissue specific (inducible) migration by specific signals from a tissue mainly in the memory immune response. These homing processes take advantage of the ability of lymphocytes to recirculate in the blood to the lymph nodes, a capacity other immune cells such as macrophages, dendritic cells do not have.

Recirculation

Nieve Cells such as naive-T enter lymph nodes from the blood supply through high endothelial venules, and search for their antigen. If no antigen is found, the cell reenters the blood vessels via HEV and recirculates again. If the antigen is found, the activated cell will instead inter the peripheral blood vessels through an allosteric change allowing them to reach the infected tissue. The molecules responsible for the recirculation process are called homing receptors, while the molecules expressed on the endothelium that allows entry of the lymphocyte into the tissue are called addresses. In the presence of cytokines the addressing receptors attract lymphocyte cells and crawl through the vessel cell walls, without entering the cells through a process called extravasation.

Constitutive Homing

Constitutive homing is only done by naive T and B lymphocytes.

Migration of leukocytes through endothelium therefore depends on blood flow, chemotactic factors, receptors present, and cytokines. Both inducible and constitutive homing work through tethering, rolling, activation, transmigration through the endothelium. Selectrons mediate this tethering, a weak binding that allows the leukocytes to slow down and roll locally. Next, integrin receptors activate the lymphocyte, and form a strong adhesion and transmigrate with help from integrins. Chemoattractant receptors work in the activation and firm adhesion processes. These intercellular adhesion molecules mediate both the adaptive homing, and allow leukocyte migration from the bloodstream to the tissue.

Selectins are membrane glycoproteins, and are Ca2P dependent lectin receptors of types selectin-P, E, and L. Some selections are expressed by leukocytes, others by endothelium, both mediate the interaction between the ligand and receptor. Selection ligands are highly glycosylated. Lymph Nodes circulating in the blood containing the selection-L protein bind the GlyCAM-1 and CD34 allow the rolling interaction. Once rolling, the lymphocyte is moving slow enough for the LFA-1 receptor to bind the chemokines bound to the extracellular matrix of the lymph node, forming the firm adhesion.

Once the firm adhesion is formed, proteins from the integrin family of proteins which play roles in adhesion in many cellular processes. These specific integrins are maintained in an inactive, low affinity state in order to only uptake lymphocytes when signaled. The conversion to the high affinity state depends on the strength, and type of stimuli, as well as the propensity to cluster together in the semi-active state also producing a strong adhesion. One important integrin in the immune system is leukocyte-function associate -1 (LFA-1) which is expressed on all lymphocytes, and interacts with endothelium ligands in an induced or constitutive manner depending on the tissue. This is thanks to the high density of specific receptors and cytokines in HVA tissues.

Lymphoid organ exit depends on the presence of absence of the antigen for the naive lymph node cell. If no antibody is found, the lymph node upregulates the chemoattractant phongoside-1-phosphate receptor which guides the lymphocyte out of the node. Instead if the antigen is found, the lymph node cell blocks the upregulation of the phingoside-1-P receptor for a couple days while the differentiated lymph node cell proliferates.

Induced homing

Leukocytes have their L-selectin protein cleaved off, making homing to lymph nodes difficult. Now, tissue specific homing consists of non lymphatic tissue homing is mediated by the upregulation of receptors for homing to inflamed tissues in peripheral tissues. Tissue specific homing have much better homing capacity for peripheral tissues than naive ones, but also the ability to select specific tissues and organs where the source of the antigen that activated them exists. This means while all lymphatic cells are activated in lymph nodes, their circulation in the blood allows localization of the response.

T cell development

T cell maturation begins in the bone marrow, but most of the process takes place in the thymus. Stem cells from the bone marrow are transported to the thymus. T-cell maturation involves the

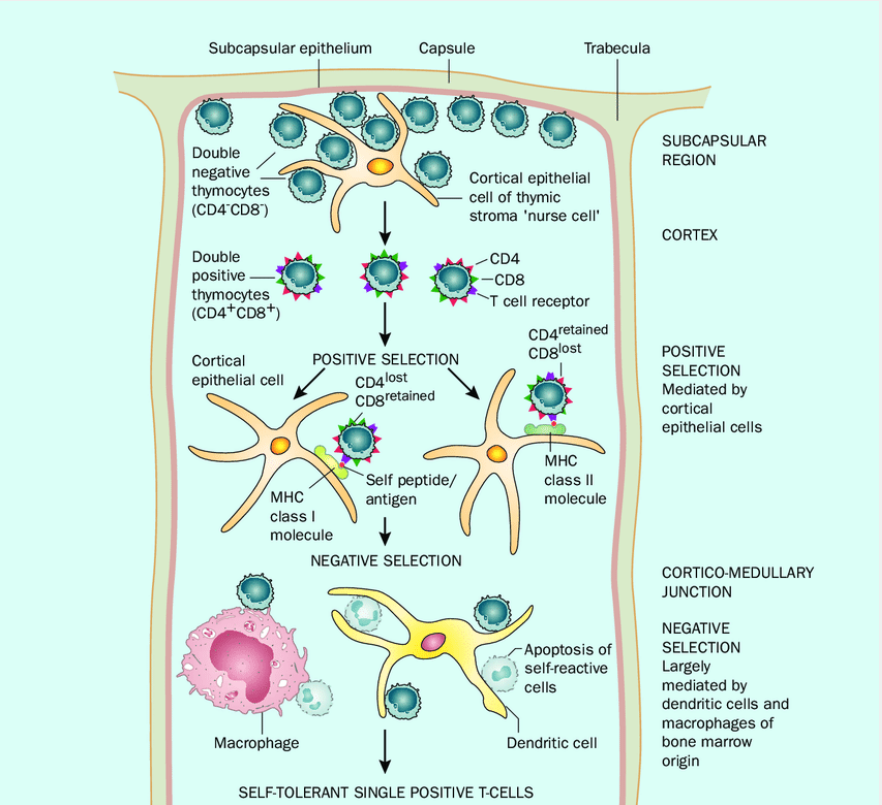
rearrangement of the germ-line TCR genes and the expression of various membrane markers. In thymus, the developing T cells are termed as thymocytes. These thymocytes proliferate and differentiate along developmental pathways that produce functionally distinct sub-population of mature T-cells. When T-cells precursor arrive at thymus, they don't express the signature surface markers of T cells as the T-cell receptors, the CD_3 complex or the co-receptors CD_4 and CD_8. After arriving in the thymus T cell precursors enter the cortex and slowly proliferate.

The differentiating T-cell passes through a series of stages that are marked by characteristic changes in their cell surface phenotype. The thymocytes early in the development lack detectable CD_4 AND CD_8. As these cells are CD_4-, CD_8-, they are termed as double negative (DN) cells. Once the DN_1 cells encounter the thymic environment, they begin to proliferate and express CD_{25} becoming C-kit$^+$, CD_{44} high and CD_{25}+, now called DN_2 cells. During the critical DN_2 stage of development, rearrangement of the genes for the TCR, γ, δ and β chain begins.

However, the TCR α locus does not re-arrange. As cells progress to DN_3, the expression of both C-kit and CD_{44} is turned off and TCR γ, TCR δ , and TCR β rearrangement progresses. Cells destined to become $\gamma\delta$ T cell, diverge at the transition between DN_2 and DN_3 and become mature. On assuming the DN_3 phenotype (C-kit$^-$, CD_{44}– and CD_{25}+), the cells halt proliferation and protein products of TCR β rearrangements are detected in the cytoplasm of these cells. The newly synthesized β-chain combines with 33-kDa glycoprotein known as the pre-T cell receptor or pre-TCR. Formation of pre-TCR activates a signal transduction pathway that has the following consequences:

- It indicates that a cell has made a productive TCR β-chain rearrangement and signals its further proliferation and maturation.
- It suppresses further rearrangement of TCR β-chain resulting in the allelic exclusion.
- It renders the cell permissive for re-arrangement of the TCR α chain.
- It induces developmental progression to CD_{4+} and CD_{8+} double positive (DP) state.

After β-chain rearrangement is completed the DN_3 cells quickly progress to DN_4, the level of CD25 falls and both CD_4 and CD_8 co-receptors are expressed. Thus, the double positive (DP) stage is a period of rapid proliferation. However, TCR α-chain gene rearrangement still has not occurred at this stage of time. An estimated 98% of all thymocytes do not mature i.e. they die by apoptosis within the thymus either because they fail to make a productive TCR gene rearrangement or because they fail to survive thymic selection. Double positive thymocytes that express αβ TCR CD_3 complex and survive thymic selection develop into immature single positive CD_4+ thymocyte or single positive CD_8+ thymocytes. These single positive cells undergo additional negative selection



and migrate from the cortex to medulla, where they pass from the thymus into the circulatory system.

Thymic selection of T-cell:

The most characteristic property of mature T-cells is that they identify only foreign antigen combined with self MHC molecules. For this purpose, the thymocyte undergoes two selection processes in thymus.

Positive selection occurs in the cortical region of the thymus.It involves the interaction of immature thymocytes with cortical epithelial cells. This interaction allows the immature thymocytes to receive a protective signal preventing them from undergoing cell death. Cells whose receptors are not able to bind MHC molecules would not encounter with the thymic epithelial cells and as a result it would not receive the protective signal resulting in their death by apoptosis, a consequence of MHC restriction.

Negative selection: The population of MHC restricted thymocytes that survive positive selection includes cells with receptors having a range of affinities from low to high for self-antigen presented by self-MHC molecule. Thymocytes with high affinity receptors are weeded out during negative selection via an interaction with thymic stromal cells. In case of negative selection, dendritic cells and macrophages having class I and class II MHC molecules interact with thymocyte bearing high affinity receptors for self MHC molecules alone. cells that undergo negative selection are observed to undergo death by apoptosis. Tolerance to self-antigens encountered in the thymus achieved by eliminating T-cells that are reactive to these antigens in the form of AIRE (autoimmune regulator), a transcription factor which expresses many self antigens not normally present in the medulla. Negative selection eliminates the self reactive receptor containing lymphocytes.

Cell Mediated Immunity (Ch5)

Cell Mediated immunity mainly deals with T lymphocytes combating intracellular microbes. Some microbes are engulfed and are able to survive in vesicles or even cytosol. Elimination of these microbes is the main function of T cell adaptive immunity.

T-cells activation is initiated by interaction of the TCR-CD_3 complex with a processed antigenic peptide bound to either class-I (CD_8+ cell) or class II (CD_4+ cell) MHC molecules on the surface of an APC as well as costimulator recognition which activate either signalling or adhesion pathways. The interaction and the resulting activating signals also involve various accessory membrane molecules on the T-cell and the antigen presenting cell. Interaction of T cells with antigen initiates a cascade of biochemical events that induces the resting T-cell to enter the cell cycle, proliferating and differentiating into memory cells or effector cells. The key element in the initiation of T cell activation is the recognition by the TCR of MHC peptide complexes on antigen presenting cells. This event catalyses a series of intracellular events beginning at the inner surface of the plasma membrane and culminating in the nucleus, resulting in the transcription of genes that drive the cell cycle and/or differentiation of T-cell.

Phases of T-Cell Response:

T-cells detect antigens of microbes and respond by increasing antigen specific t-cells and conversion of naïve to effector T cells. This activation is mediated by transport of antigens to peripheral lymphoid organs where naïve T cells recirculate along with other stimuli (example IL-2). Upon activation the T cells begin to secrete cytokines some of these stimulate clonal expansion. Some of these T cells produced from colonial expansion will undergo differentiation into effector T cells. Others will continue to circulate the system for months and are called memory T cells. After some time, the stimulus for activation is eliminated and the T cells are inactivated again.

Antigen Recognition and co-stimulation:

T-cell response requires:

- TCR recognizing MHC associated peptide antigens
- CD4 and CD8 coreceptors which recognize MHC molecules
- Adhesion molecules to strengthen TCR-APC binding, mainly LFA1 to ICAM1
- Costimulatory receptors recognizing secondary signals from APC, mainly CD28 to B7-1 or B7-2
- Cytokines which amplify the T cell response and direct it along various differentiation pathways

Non-antigen receptor molecules are called accessory molecules and serve for signaling or Acacian. The TCR plus CD4 for CD8 coreceptors recognize MHC plus peptide to make the initiating contact.

- CD4 → Cytokine producing T → Recognize peptides of ingested extracellular microbes → MHC-II
- CD8 → Cytotoxic Lymphocyte (CTL) → cytoplasmic microbes' peptides → MHC-I

The specificity of CD4 and 8 make sure the vesicular and cytoplasmic microbes elicit the correct immune response. T activation requires two or more TCR and Cora scepters to be stimulated for several minutes. This activation is by TCR associated proteins which form the TCR complex and CD 4/8, coreceptors.

TCR Complex: CD3 (3 chains), ζ dimer for signaling, TCR (a and b) for antigen recognition

Adhesion molecules on T cells recognize APC ligands and stabilize T-APC binding. Integrins such as LFA1 - which binds ICAM1- are kept in a lower affinity state unless cytokines from the innate immune system signal the conversion to high affinity state. Once in this high affinity state, LFA1 induces other LFA1 units to convert in a cooperative way forming a positive feedback loop. Integrins therefore play an important role in enhancing T cell response.

Co-stimulator's provide stimuli to T cells allowing them to be fully activated. B7-1 (CD80) and B7-2 (CD86) the most commonly expressed co-stimulators on APC and increase when APC encounters microbes.

- B7 is recognized by CD28 found on virtually all T-cells, and signals activation together with signals from MHC-TCR binding. Activation is not possible without B7-CD28 binding
- Another costimulatory, CD40 ligands (CD154) on T cell bind CD40 on the APC inducing APC to make more B7 receptors, as well as secrete IL-12 for T differentiation in lymph organs.

This co-stimulation requirement is needed in order to prevent T activation to harmless foreign substances. Vaccines require adjuvants to activate APC to express costimulatory, in order to stimulate the T cell response or vise versa. Medicine also take advantage of CD28 homologues in order to limit or terminate an immune response.

Inhibitory Receptors of T Cells: Inhibitory receptors are critical for limiting and terminating immune responses. Two important inhibitory receptors: CTLA-4 and PD-1 are structurally related to CD28. CTLA-4,

like CD28, recognizes B7-1 and B7-2 on APCs, and PD-1 recognizes different but structurally related ligands on many cell types. Both CTLA-4 and PD-1 are induced in activated T cells, and function to terminate responses of these cells. CTLA-4 also plays an important role in the suppressive function of regulatory T cells.

CD8 Activation is stimulated by MHC-I peptides and co-stimulators, as well as sometimes helper T cells. Strangely, CD8 often require cross presentation of the antigen by dendritic cells to be activated. Activation may also require parallel activation of CD4 helper cells: CD8 and 4 are activated by the same viral peptide, next CD4 secrete activating cytokines for CD8. This phenomenon of co activation explains why people with HIV -which kills CD4- are unable to defend against many viral infections related to CD8 as well.

Biochemical Pathways of T Activation:

Antigen recognition activates several biochemical mechanisms that lead to T cell responses, including the activation of enzymes such as kinases, recruitment of adaptor proteins, and production of active transcription factors. The CD4 and CD8 coreceptors facilitate signaling through a protein tyrosine kinase called Lck. CD3 and ζ contain motifs, each with two tyrosine residues, called immunoreceptor tyrosine-based activation motifs (ITAMs) which are the site of phosphorylation. So CD3 activates Lck, which phosphorylates ζ, phosphorylated ζ serves as a docking site for ZAP-70 which has three major pathways, NFAT, Ras/Rac-MAPK, AP-1 activation.

Nuclear factor of activated T cells (NFAT) is a transcription factor present in an inactive phosphorylated form in the cytoplasm of resting T cells. NFAT activation depends on Ca^{2+} ions in the cytosol. This signaling, initiated by ZAP-70, is the phosphorylation and activation of phospholipase Cγ (PLCγ), which hydrolyzes plasma membrane inositol PIP2. One by-product of PLCγ-mediated is IP3 which binds to IP3 receptors on the ER membrane releasing Ca^{2+} from the ER, thereby raising the cytosolic Ca^{2+} concentration. This increased Ca results in opening of pores on the PM in order to bring in more calcium to replace that lost from the ER. The elevated cytoplasmic Ca^{2+} leads to activation of a phosphatase called calcineurin. Calcineurin removed phosphate from NFAT, so it can enter nucleus and bind regulatory regions, including genes encoding growth factor IL-2 and IL-2 receptor.

Ras/Rac–MAP kinase pathways: initiated by ZAP-70–dependent phosphorylation and accumulation of adaptor proteins at the plasma membrane, leading to the recruitment of Ras or Rac, and their activation by exchange of bound GDP with GTP. GTP bound Ras/Rac are active form activate distinct MAP kinases. The terminal MAP kinases in these pathways, called extracellular signal–regulated kinase (ERK) and c-Jun amino-terminal (N-terminal) kinase (JNK), respectively, induce the expression of a proteins c-Fos and the phosphorylation of another protein called c-Jun. c-Fos and phosphorylated c-Jun combine to form the transcription factor **activating protein 1 (AP-1),** which enhances the transcription of several T cell genes.

Nuclear factor-κB (NF-κB): Activation of the θ isoform of the serine-threonine kinase called protein kinase C (PKCθ), leads to the activation of NF-κB. PKC is activated by diacylglycerol (DAG) generated by PLC-mediated hydrolysis of membrane inositol lipids. NF-κB exists in the cytoplasm of resting T cells in an inactive form, bound to an inhibitor called IκB. TCR- induced signals downstream of PKCθ activate a kinase that phosphorylates IκB and targets it for destruction. As a result, NF-κB is released and moves to the nucleus, where it promotes the transcription of several genes.

PI-3 Kinase: Is also activated, phosphorylating PIP2 to generate PIP3 which can activate AKT. AKT stimulates many anti apoptosis genes.

Functional Responses of T to antigens and costimulators

Secretion of cytokines and expression of cytokine receptors is activated especially in CD4+ cells in response to antigen and costimulators. CD4 for first produce IL-2 and IL-2 receptors within hours. IL-2 receptors is a dimer in its inactive state, and addition of another monomer makes it higher affinity active state. IL-2 stimulates survival and proliferation. CD8 cells recognizing antigens do not secrete significant amounts of IL-2. The principal functions of IL-2 are to stimulate the survival and proliferation of T cells.

Clonal expansion occurs within 1-2 days of antigen recognition and costimulators, resulting in large amount of antigen specific clones. This rapid expansion is required to keep up with rapidly multiplying microbes within the system, CD8 cells expand much more rapidly than CD4 and both only recognize a few peptide antigens.

Differentiation of naïve T cells into effector cells

CD4 helper T cells differentiate to produce source molecules and cytokines which activate phagocytosis and B-lymphocytes, most important of these is the CD40L ligand which activates CD40 on dendritic cells via MHC. CD4 helpers differentiate into subsets of effector cells producing distinct cytokines; TH1, TH2 or TH17.

- Th1: Secrete IFN-Y, which induces phagocyte ingested killing of intracellular pathogens, and antibodies isotypes which promote phagocytosis
- Th2: Produce IL-4, 5 and 13 which all stimulate eosinophil mediated immunity against helminths and induce mucus secretion. These Interleukins also activate macrophages Via the 'alternative activation pathway'
- Th17: Secrete IL-17, 22 which promote inflammation, and defense against bacteria

Differentiation is controlled by master cytokines:

- Th1 ⟶ IL-12 (Positive feedback) and IFN-Y (NK cells)
- Th2 ⟶ IL-4
- Th17 ⟶ Il-6, 1, 23 and TGF-B

CD8 cells instead become cytotoxic lymphocytes (CTL) and are discussed next chapter.

Development of memory T cells

Memory cells survive even after the infection is eradicated and antigen is no longer present. Certain cytokines, including IL-7 and IL-15, which are produced by stromal cells in tissues, may serve to keep memory cells alive. Memory T cells may be rapidly induced to produce cytokines or kill infected cells on encountering the antigen that they recognize. These cells do not perform any effector functions until they encounter antigen, but once activated, they respond much more vigorously and rapidly than do naive lymphocytes. Memory T cells can be found in lymphoid organs, in various peripheral tissues, especially mucosa and skin, and in the circulation.

Migration of effector T to site of infection:

Effector T cells migrate to the site of infection due to high expression of adhesions and cytokine receptors for ligands expressing an infected endothelium. Naïve T cells remain in the lymph nodes due to high expression of L-selectin, LFA-1, CCR7 binding with L-selectin ligand, ICAM-1 and CCL19/21 in the lymph nodes respectively. Activated T undergo homing to sites of infection because they express E and P selectin ligands, LFA-1 or VLA-4 and CXCR3 which bind to E/P selectin, ICAM-1 and VCAM-1 and CXCL10 located in the infected endothelial cells.

Selectins initiate the rolling action discussed, which LFA→ICAM or VLA→VCAM initiate stable adhesion. CCR7→CCL19 and CXCR3→CXCL10 activate integrins and chemotaxis which help in the homing process. Homing of lymphocytes to infected tissue or back to lymph nodes is independent of antigen recognition, but lymphocytes who recognize microbes at each site are much more likely to stay there.

The phospholipid sphingosine 1-phosphate (S1P) plays a key role in the egress of T cells from lymph nodes. The levels of S1P are higher in the blood and lymph than inside lymph nodes. S1P binds to and thereby reduces expression of its receptor, which keeps the expression of the receptor on circulating naive T cells low. When a naive T cell enters the node, it is exposed to lower concentrations of S1P, and expression of the receptor begins to increase.

Lymphocytes containing any antigen will go to any infected tissue, but once inside the ones who are able to recognize a microbe are selected to stay, as they express increasing VLA whereas those who do not encounter a matching antigen will not be selected to stay and continue circulating. Contrasting with activation of naive T which requires costimulators, differentiated effector T are less dependent on costimulators. Activation is therefore limited to lymphoid organs, while functions of effectors can be in any tissue.

Decline of the Immune Response

Once an infection is cleared and the stimuli for lymphocyte activation disappear, many of the cells that had proliferated in response to antigen are deprived of these survival signals. As a result, these cells die by apoptosis.

Numerous mechanisms have evolved to overcome the challenges that T cells face in the generation of a useful cell-mediated immune response:

- Naive T cells need to find the antigen. This problem is solved by APCs that capture the antigen and concentrate it in specialized lymphoid organs
- The correct type of T must respond to antigens from the endosomal and cytosolic compartments. This selectivity is determined by the specificity of the CD4 and CD8 coreceptors for class II and class I MHC molecules
- T cells should respond to microbial antigens but not to harmless proteins
- Antigen recognition by a small number of T cells must lead to a response that is large enough to be effective. This is accomplished by robust clonal expansion.
- The response must be optimized to combat different types of microbes. This is accomplished largely by the development of specialized sub- sets of effector T cells.

Differentiation

The high concentration of CD4 and 8 cells is needed in large amounts at the time of infection, but not constantly after. For this reason, inhibitory receptors such as CTLA-4 and PD-1 are activated

after some time when the response is no longer needed. CTLA-4 is expressed after days of t cell activation, and has a higher affinity for B7 than CD28, and therefore removes the signal for activation after some time. PD-1 interaction with PDL1 on the APC creates a signal cascade which downregulates cytokine production, proliferation, and cell survival.

Cytokines

Innate lymphocytes

While adaptive lymphocytes need TRC, co-stimulator and cytokines to be activates, innate lymphocytes only need co-stimulators and cytokines. One population of innate lymphocytes is NK cells, this is the historically known population. We are now aware of several new subsets such as ILC1, ILC2, LTi-like, and ILC3, where act similarly to T-helper cells, with the huge difference that they do not need antigen recognition. These innate lymph nodes are named based on their secreted cytokine, and have the same lineage defining cytokines as t-helpers.

NK cells and ILC1 are similar in that they both produce INF-Y, but ILC1 in response to IL-12 is not able to kill the cells like NK can. This difference is pronounced by the location of ILC1- in the tissue. Since they ar at the site of infection, they can be activated faster than circulating NK cells. ILC2 is homologous to Th2 cells, and is also important in helminth parasite infection. ILC2 are activated by IL25 and 33, and produce Il-5 which activates eosinophils. ILC2 also secrete IL-13, important in activation of phages and mucose production.

ILC3 and Lti-Like can be compared to Th17 and Th22 producing IL-17 and 22. IL23 activates both of these cells through activation of RORyt master transcription factor, the same for Th17 and 23 activation. IL17 produced by these cells activates neutrophils, releasing granules. IL-22 is instead important in the protection and renewal of epithelial cells. LTI-like cells are important in embryology in the generation of lymph nodes.

ILC development

Differing from T cells, ILC cells differentiate in the bone marrow from multipotent cells, into differentiated cells. Although most transcription factors are conserved between ILC and T cells, Id2 is able to block the function of E proteins in the bone marrow, a protein required for T development before leaving the bone marrow.

The activation of ILC is not regulated epigenetically such as in t-helpers, therefore right when ILC encounters a parasite it can be activated, while T helper requires epigenetic modifications of the chromatin.