W1

Immunology Overview

Special immune cells are produced and/or mature in the organs of the lymphatic system:

- Thymus
- Lymph nodes
- Bone marrow
- Spleen

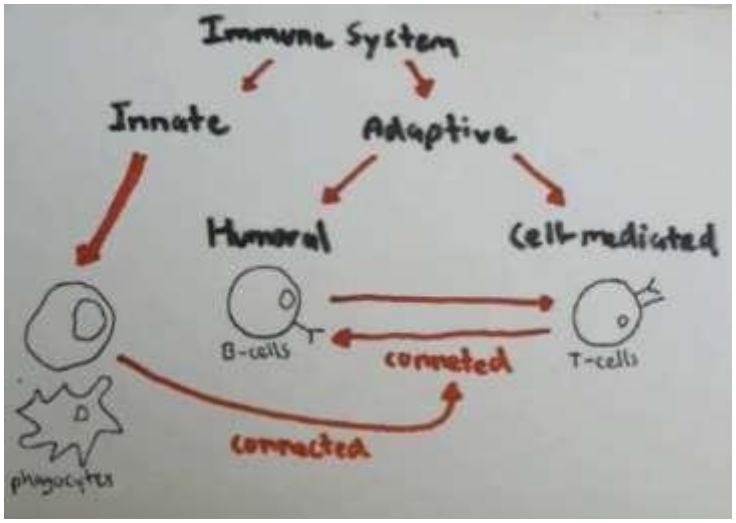
A phagocyte can differentiate between self and non-self-bacteria.

The immune system can be divided into two:

- Innate: Quick response. First line of defense. Mainly macrophages, dendritic cells, mast cells, complementary protein, chemical mediators.
- Adaptive: Delayed, second line in defense and is antigen-specific. B-cells (secrete antibodies), T-cells which differentiate into T-killer cells and T-helper cells. B- and T-cells have to work together in order to destroy the pathogens.
    - Humoral: Mainly B-cells.
    - Cell-mediated: Mainly T-cells.

The innate immune system is connected to the adaptive immune system by T-cells.

Antibodies are proteins that are able to bind onto antigens on different pathogens. The immune cells, such as phagocytes, can the easily eliminate the pathogens.



When a pathogen invades the body, the innate immunity kicks in. The phagocytes will try to destroy and consume the pathogen. If this fails, the adaptive immune system kicks in. The phagocytes will present the antigens of the pathogen to the T-cells. The T-cells will differentiate into T-killer cells which will destroy the pathogen or into T helper which will activate B-cells that will secrete antibodies to help and destroy the pathogen.

histamine which causes vascular dilation and increase vascular permeability (endothelial cells will contract and leaves gaps). Immune cells migrate to infected tissues. Macrophages will also recognize PAMP and secrete cytokine (particularly TNFα and interleukin-1). This causes inflammation and triggers repair. Cytokines causes leukocytosis (accumulation of white blood cells) and fever.

Part II – Inflammation

Cytokines fibroblasts to proliferate and stimulate collagen synthesis, which causes repair of tissues.

Neutrophils and macrophages bind to PAMP and engulf pathogens.

Complement proteins with antibodies can cause opsonization or lysis of pathogen:

- Opsonization: Coating of pathogen which allows macrophages to detect and destroy pathogens more easily.
- Lysis: Cell burst

Four Greek words that characterize inflammation: rubor (redness), calor (heat), tumor (swelling), dolor (pain) (+ loss of function, due to chronic inflammation)

Lecture

The primary removal of worn out cells and tissue debris is carried out by macrophages.

Viruses have a much faster evolution rates compared to humans which challenges our immune system. They can develop strategies to circumvent our immune system to fast for us to handle.

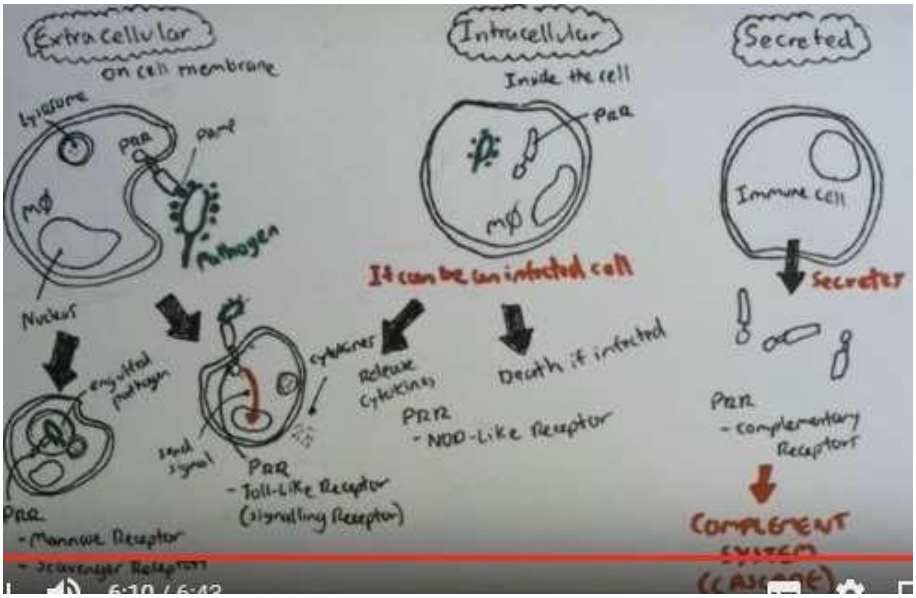
We need not to have an overactive immune system because it requires a lot of energy. We don't want too much of the bi products of the immune cells, such as cytokines (signaling molecules) as they can kill self-cells. Tissue destruction can occur from prolonged exposure to cytokines, so we want to avoid chronic inflammation. We need a balance between reactivity and over reactivity.

A good immune response is a selective one. We don't want a reaction all the time. Such as a reaction to self-organs and innocuous substances since they cause autoimmunity and allergy.

Intracellular pathogen = infecting and living inside the cell
Extracellular pathogen = infecting and living outside the cell

We need to be able to distinguish between intra- and extracellular pathogens as they give rise to different responses. Viruses are always intracellular pathogens.

Effector cells only produce inflammatory mediators during inflammation. The immune cells get to the infected tissues through the blood. When a bacterium enters the skin, it is recognized as foreign. Then they are engulfed my macrophages. Cytokines are then released which causes vasodilation. Immune cells then enter the infected tissue through the blood vessels. More immune cells produce more cytokines that strengthen the reaction. This causes redness, heat, swelling, and pain – therefore inflammation.



Innate immunity (Scavenger receptors)

Scavenger receptors are found on many cells and are usually known for their role in the cardiovascular system. They bind on low density lipoproteins (bad cholesterol, LDL) and float around in the blood flow. When a scavenger receptor on a macrophage bind to a LDL it will become a foam cell and will then attach on the vessel walls. Accumulation of these foam cells will clog the blood vessel and cause atherosclerosis. Scavenger receptors are also PRRs and can bind PAMPs.

Scavenger receptor A-1 (SR-A1) or SR-A2 and MARCO can be categorized to Class A scavenger receptors, because they have a similar lower domain and have a collagen domain on the top which allow them to bind to the same things. CD36 is a class B scavenger receptor. CD36 bind HDL (high density lipoprotein, good cholesterol). SR-C1 in class C, CD68 in class D, LOX-1 in class F, and SCARF in class F.

Class A and class B scavenger receptors are expressed on macrophages and help in phagocytosis of pathogens. SR-A1 and SR-A2 bind to the cell walls, whereas CD36 also bind to diacylglycerols (long chain fatty acids) of pathogens. Scavenger receptors works as a co-receptor to Toll-like receptors. When TLRs bind to a certain pattern of a pathogen it needs the help of a scavenger receptor. When a TLR binds to a PAMP with the help of a scavenger receptor, it will initiate a cascade of events in the cytosol, where the final product is a transcription factor. The TFs enter the nucleus and induce expression of cytokines that will then secrete and assist in the immune response, fx attracting more immune cells to the site of infection.

When a PAMP of a pathogen bind to SR-A1 of a macrophage it will be engulfed by phagocytosis. It will then be packed into a phagosome. There is also a lysosome present in the cell which consist of toxic acidic compounds that eliminate pathogens. The lysosome fuse with the phagosome and releases it content. Now it is called a phagolysosome and will destroy the pathogen.

When a dendritic cell interacts with a naïve T cell it presents the antigen on top of MHC I or II molecule. During the process of activation, we also have upregulation of B7 (CD80/CD86) that are expressed by activated dendritic cells. B7 interacts with CD28 on the naïve T cell (a T cells that has not been activated before). During this interaction, the naïve T cell can get different phenotypes, depending on the different types of cytokines secreted by the dendritic cell that it is interacting with.

If the dendritic cell produce the cytokines IL-12p60, IFN-α, or IFN-β it can give rise to CTL or Th1. CTL and Th1 will produce the cytokine IFN-γ. If the dendritic cell produces IL-23, IL-1β, or IL-6 it will propagate production of Th17. Th17 produces the cytokine IL-17A. If the cytokine profile of the dendritic cell is no IL-12p70, TSLP, or IL-4 it will give rise to Th2. Th2 produces the cytokines IL-4, IL-5, and IL-13. When the immune suppresses phenotype is active, the cytokine TGF-β is expressed by the dendritic cell, which activates Treg. Treg produce IL-10 or TGF-β.

We have 4 different types of adhesion molecules that mediate extravasation of leukocytes into the tissues:

- Vascular addressin (CD34)
- Selection
- Integrin
- Immunoglobulin-like molecule

Extravasation = diapedesis

Important cytokines produced by macrophages:

- IL-1β: Fever and production of IL-6, induction of acute-phase protein production.
- TNF-α: Fever and mobilization of metabolites. Septic shock when it is present in the systemic circulation. Induction of acute-phase protein production
- IL-6: Fever, induction of acute-phase protein production and complement system.
- CXCL8: Diapedesis. Important chemotactic factor (attracts neutrophils to the site of infection).
- IL-12: Induces differentiation of naïve $CD4^+$ cells into Th1 cells (immunity against intracellular microbes).

Chemokines are proteins and there two subtypes. Attracts all leukocytes. Secreted into the extracellular fluid and can attach to the extracellular matrix that governs the tissue and endothelium cells. Chemokines also bind to chemokine receptors which mediate signals.

CXCL8 gives rise to the recruitment of neutrophil from the bloodstream into the tissue site (important that we know this). CXCL8 are produced when epithelial cells are activated via PAMPs.

Neutrophils have a life span below 2 days and die by apoptosis and get eaten by macrophages.

IFN-α and IFN-β induce resistance to have viral infections in nearby cells. While having one infected cell by a virus, there is a secretion of interferons. IFN-β work in a paracrine manner (neighboring cells), which causes the neighboring cell to be protected against the virus. IFN-β also works in an autocrine manner on the host cell itself.

NK cells have specific receptors that can sense if a host cell is infected. During infection, the infected cell will have an upregulation of a specific protein called MIK that bind to a specific

cytidine deaminase (AID) to access the switch regions. As RNA polymerase II transcribes the sterile transcript, the repetitive elements in the switch region induce the behavior in the polymerase that allows AID to deaminate single stranded cytidine residues in the DNA and convert them randomly to uracils. Through the action of two other enzymes uracil-DNA glycosylase (UNG) and apyrimidinic endonuclease (APE1), these uracils are eventually converted into single stranded nicks in the DNA on both strands. This happens in all the switch region activated in the B cell. Thus, this creates multiple breaks in the DNA. Because breaks have been introduced in great distance form one another, the double strand break machinery joins the two switch regions and excises all of the DNA located between the two damaged regions. The damaged switch regions will have been spliced together at some random point. The rearranged VDJ segments is now places a few kb upstream of a new constant region. This now makes the transcription initiated from the V region promoter to generate a transcript encoding the original variable region but now in the context of a different constant region. When the mRNA is fully spliced, the intervening switch regions are removed, leaving only the segment encoding a fully functional heavy chain. The activated B cell will then begin the secrete the antibody of the new isotope class.

W5

Introduction to intracellular signaling

Many of the enzymes responsible for intracellular signaling are kinases and fx scaffolds/adaptors.

Effectors of signaling:

- Protein kinase: Adds negatively charged phosphate group to Ser/Thr/Tyr (Ser/Thr are very similar, Tyr is very different.) residues in humans. Specificity! The kinases that phosphorylate Ser also phosphorylate Thr but not Tyr. Some have dual specificity (all three, but not abundant). Phosphatases remove the phosphate group and also have a specificity like the kinases.
- Lipase: Hydrolyzes ester bonds in lipids. In signaling this is often membrane bound lipids that are hydrolyzed to form diacylglycerides. These can recruit enzymes to the membrane or diffuse through the cell to activate molecules that are far away from the receptors.
- Ubiquitin ligase: Attaches ubiquitin to Lys residues in proteins. You end with a poly ubiquitin chain on the peptides that allow other proteins to recognize it and bind the chain. Because the ubiquitin is linked to K63 instead of K48 (that tags it for protein degradation) then the protein will not be degraded but is a way of conveying signaling.
- 2^{nd} messengers: Ca^{2+}, IP3. Small molecule effectors that can easily diffuse away in the cell and activate molecules that are far away from the receptor complex. Calcium influx into cell, which can bind a molecule inside the cell which can lead to signal amplification.

When a receptor binds its ligand, something has to happen. Kinases can be a part of membranes and are in the cytoplasmic region the receptors. When not bound, they are monomers and not activate because they are not in close vicinity to their substrate. When the receptors bind a ligand, the two monomers go together and can phosphorylate each other and activate. Not all receptors contain kinases as an integral part of the protein but have a kinase non-covalently bound in the cytoplasmic part of the receptors but the output is the same.

There are three major ways for DCs to uptake antigens:

- Receptor-mediated endocytosis: Endosome is afterwards fused with lysosome to degrade the microbe.
    - Complement receptors that interact with C3b on the surface of pathogens
    - Fc receptors that interact with Fc region of antibodies coating microbes
    - C-type lection that interact with sugars on microbes¨
- Micropinocytosis: Extracellular matters in the DC which are degraded.
- Infection by intracellular microbes: Usually lead to MHC class I presentation. There can also be cross-presentation. If the virus escapes the endosome of the infected cell, it can still be presented on MHC class I. One DC can transfer antigens to nearby DCs, which also involve MHC class I molecules (usually takes place in the lymph nodes).

Tissues factors present at the site of infection and which cytokines they produce, can to some extend influence how the DC is matured and which type of co-stimulatory molecules and cytokines are expressed by it. Depending on which PAMP, then the DC will have different phenotypes. It will produce specific cytokines related to the PAMPs.

When the DC is activated, it presents peptides on both MHC class I and II molecules, together with B7 and express cytokines, depending on the microbe. When the DC reaches the lymph nodes, it starts to interact with naïve T cells. The given TCR that can interact with the peptide:MHC complex can then become activated. The naïve T cells needs to interact with the peptide:MHC complex, the B7 complex (through CD28), and cytokines, in order to become activated. The specific cytokines will direct it to have a specific effector function.

Inactivated DCs in tissues are usually round but during its activation its getting dendrites. Different types of T cells can interact with the same DC at the same time. The way that the activated DC can migrate to the draining lymph node is via the expression of the chemokine receptors CCR7. CCR7 recognizes the chemokines CCL19 and CCL21 that are deposited along the lymphatic vessels. Then the DC can roll from the site of infection to the lymph node. Macrophages will stay at the site of infection and not migrate to the draining lymph nodes.

During the process from immature DC to mature DC is induction of antigen presentation, upregulation of co-stimulatory molecules (B7 and CD40 on some types of DCs), upregulation of CCR7, and specific cytokines.

Within the draining lymph nodes, we have different zone. Within the T cell zone, we have stromal cells that produce CCL21 which enables the DC to enter the lymph node because the CCR7 on the DC will recognize CCL21. When the DC starts to mature in the lymph nodes, it starts secreting CCL18 and CCL19. These can attract T cells from the blood into the site of the T cell zone so they can interact with the DCs. Most of these T cells will be naïve.

Activation of T cells in lymph nodes

HEV: High endothelium vessels. Blood structures through which T cells can enter the lymph nodes. If the T cell does not encounter an MHC:peptide on an APC, it moves out again via cortical sinuses, into the medullary region and out through the efferent lymphatics. Activated T cells will not leave the lymph nodes in the first four days.