# Innate Immune System

## Complement system: proteins attached to the bacteria, is initiated by:

* **Lectin pathway:** mannose-binding lectin (MBL) binds to the mannose residues or these carbohydrate sequences found on the pathogen surface.
* **Classical pathway**: protein C1q binds to the pathogen surface or to antibodies found on the surface of the pathogen.
* **Alternative pathway:** C3 undergoes spontaneous hydrolysis and binds to other complement proteins at the surface of the pathogen.

All these 3 pathways result in generating **C3 convertase**, which cleaves C3, leaving a soluble C3b which is bound to the surface of the pathogen.

C3 convertase results in 3 effects:

* Fragments that cleave such as C3a and C5a are inflammatory signals which signal to the cell that something is happening at the site of infection, bot recruit and activate immune cells.
* It helps cells phagocytose or eat the pathogens because immune cells have receptors for C3b.
* It results through a series of additional protein interactions which leads to formation of a **membrane-attack complex (MAC)** which disrupts cell membrane and cause cell lysis.

*Complement system: security monitoring and land mines*.

Complement signals to the immune cells in the blood and 3 major events happen:

1. Increase vascular permeability so proteins and cells can move from the blood to the site of infection.
2. Activate cytokines.
3. Chemokines, subset of cytokines, induce cell migration

# Cells of the Innate Immune System

**Neutrophils** *(frontline soldiers)* destroy pathogens:

1. Antimicrobial peptides
2. Enzymes
3. **Neutrophil extracellular traps (NET)**: formed by long strands of DNA mixed with anti-microbial peptides, initiated by the generation of reactive oxygen species (ROS); the nuclei lose their lobules; the nucleic acid material fills the cytoplasm mixing with the neutrophil granules which contain the anti-microbial peptides and ROS. Then the cells release the nets nuclei acidic material which kills the bacteria through concentrating anti-microbial peptides which are bound to the sticky DNA.

With neutrophil death, there is local tissue damage, not only to these factors that hurt the bacteria but also damage the local tissue where inflammation is occurring.

* **Danger-associated Molecular Patterns (DAMPs)**: cells and tissue release, a way for the body to recognize that there’s been damage done to the tissue thus many DAMPs discovered have been found to have intracellular origin. Each of the DAMPs activates known cellular receptors DAMP interacts with receptor it induces a cell signaling cascade that changes transcriptional regulation in the cell activating or increasing inflammatory state of the cell. DAMP can activate macrophage which secrete cytokines: *TNF-alpha, IL-6*; that recruit additional neutrophils to the site and they begin phagocytose pathogens around them.
* **Pathogen-associated Molecular Pattern** (PAMPs): signals from the pathogen itself activate macrophages or DCs. Detect pathogens living in the extracellular space or are phagocytosed. TLRs composed of multiple leucine-rich repeats useful to recognize PAMPs. TLRs membrane associated proteins; some located at surface of the cell or located on endocytic vesicles where they survey degraded contents of pathogens taken up by endocytosis. **TLR-9:** DNA with unmethylated CpG once the genome has been degraded in the lysosome.

Two classes of receptors that can detect pathogens (viruses) exist and are replicated in the cytosol and signal their presence to the immune system:

* Members of the nucleotide oligomerization domain family or **NOD proteins.** NOD 2 can detect bacterial proteoglycans of intracellular bacteria. NOD2 detects its ligand muramyl diptide, it sends the signal to the nucleus to activate transcription.
* Intracellular receptor proteins that contain an RNA helicase domain and caspase recruitment domain (car domain) **RIG-I** recognize double-stranded RNAs component of many RNA viruses. Send signal to the nucleus but unlike TLRs or NODs activate production of type 1 interferons.

**Macrophages** *recognize DAMPs and PAMPs, cell resident act as sentinel.* No need to be recruited, first cell to respond.

*TLRs and NOD receptor act as radars and alert the sentinels.*

# Adaptive Immune System

**DCs***, spies of the immune system*, activated by complement, DAMPs or PAMPs.

They can phagocytose pathogens, they capture pathogens, to transport them to other cells in the lymph node (basis of the immune system) to report on what’s happening at the site.

Lymphatic system formed of vessels of endothelial cells and found in nearly all tissues. Lymphatic vessels carry extracellular fluid or drain the fluid such as plasma that escape from the blood circulation and re-introduced later to the circulatory system. They also serve as a conduit to immune cells like DCs. As DCs migrate toward the lymph node: afferent to efferent lymphatic vessel:

* Low molecular weight antigens, < 70 kilodaltons:

1. enter the fibroblastic reticular cells (FRCs) which lead to B-cell follicle where they are captured by follicular dendritic cells (FTCs).
2. Alternatively, they can be carried through the lymphatic vessels and captured by digitating macrophages within the wall or sampled by these medullary sinus DCs or lymph node resident DCs.
3. They can move through the endothelial venules back to the blood circulation or the efferent lymph node.to next lymph node.

* High molecular weight antigens: do not follow small ones due to mechanical filtration with FARC conduits, sampled by resident subcapsular sinus macrophages or DCs.
* Particles and immune complexes (exosomes, macrovesicles, apoptotic bodies, viruses or bacteria), < 1um, captured by subcapsular sinus macrophages, DCs.
* Transporting or activated DCs become more sensitive to chemokines such as CCL21, migrate toward chemical gradient. Can interact with lymph node stromal cells and move toward T cell zone.

Parts of pathogens: antigens presented by DCS in MHC molecules.

Besides the antigen specific interaction**, signal 1 (MHC and TCR**), T-cell needs also **a co-stimulatory signal (signal 2),** to know that it is a foreign peptide MHC combination, not to generate autoimmunity. **CD40** on DC gets upregulated when DCs are activated by AMPs or DAMPs through TLR signaling. Signal 3 is secreted by cytokines such as IL-12. DCs present antigens in MHC II molecules to CD4+ T-cells.

**CD4+ T-cells are like the government:** they instruct and give support to other cell types to fight the pathogen.

Each T-cell has unique TCR which recognizes a unique epitope or antigen bound to the MHC (7 to 14 amin acids) on the DC.

Besides low molecular weight antigens getting into follicular DCs, high molecular weight antigens also make their way to B-cell zones **the subcapsular sinus macrophages** act as a conduit and present antigens themselves. Both subcapsular macrophages and **the follicular DCs** (*computer*) are decorated with antigens. They can bind with the B-cell receptor and become activated. BCR are antigen-specific, and when B-cell bind to their antigen this activates B-cell.

Compared to TCR:

* BCRs are dimeric (2 regions that can bind to the same epitope)
* They directly bind to the epitope or antigen (no need for another cell)

Once bound, this signals to the B-cell to internalize this antigen and digest it, which leads to the loading of the antigen on MHC molecules of the B-cell: APC.

Once B-cells will migrate towards the T-cell zone, and activated CD4 T-cells (activated by DCs) will migrate to B-cell zone. If CD4 T-cells express the right T-cell receptors for this antigen presented by B-cells: signal 1 to the T-cell, if T-cell was activated, it will express a co-stimulatory molecule (CD40-L) which acts as signal 2 and also secrete cytokines (IL-4,5,6) which will activate B-cells and CD4 T-cells which both them start proliferating. The cells activated differentiate into T cell helper.

**Antibody (missiles, humoral activity)**:

* Y-shape, two antigen-binding sites called **FAB portions of the antibody**. Constant region conserved between antibodies at the bottom of the Y: Fc portion; 150Kdalton.
* Secreted antibodies leave the lymph node, where they are produced and travel to into the circulatory system. Can leave it because of the dilated vessels at site of inflammation.
* The antibodies mediate immunity in 3 ways:

1. **Neutralization**: epitopes on surface of pathogens can get saturated preventing entry of pathogens further into tissues or cell.
2. **Opsonization** (increase of phagocytose): macrophages and neutrophils have receptors for the Fc of the antibody (Fc receptors), they have also CR1 receptors that bind to C3b; when cross-linked by many antibodies on pathogen; this increases the ability for them to phagocytose the pathogens.
3. **Enhances complement activation**: antibody will react with complement enhancing opsonization and lyses some bacteria.

*B-cells: engineering war department.*

# Viral Immune Response

* Viruses are much smaller than bacteria (100x), nm size. Genetic material encapsulated by protein capsid, coated by lipid membrane. Don’t have machinery to reproduce themselves.
* They fuse with the membrane, unload their load, fuse with nucleus machinery to trigger replication and assemble to create new viruses that bud off and infect other cells.
* DNA or RNA viruses can bind to different receptors; initiate TLR signaling and activate antigen presenting cells.
* Immature DC reside in peripheral tissues sample environment for antigens or viruses using **macropinosomes**. They take out pieces of the virus. They transfer that information into the lymph node and activate cells.

**Routes of antigen processing for DCs for CD4+ T-cells:**

* Receptor-mediated phagocytosis: engulf entire pathogens.
* Macro-pinocytosis: eats up antigens of bacterial fragments.

**Routes of antigen processing for DCs for CD8+ T-cells:**

* Viral infection of DCs
* Cross-presentation after antigen has been taken up either though fingers, or macropinocytic uptake.
* Transferred by other DCs to resident DCs through vesicles.

**CD8+ T-cell or cytotoxic cells or CTL** (*special forces*) and APC interaction (case of virus infection):

* Signal 1: presented by MHC I with the antigen that interacts with TCR on CD8 +T-cells.
* Signal 2: co-stimulatory interaction **CD80/CD86 on DCs and CD28 on CD8 T-cell**
* Signal 3: cytokines secretion to support activation of T-cells, proliferation stimulated by secretion of IL-2 and differentiation.
* How CD8+ T-cells kill: perforin, granzymes and granulysin.
* Viruses have evolved mechanisms to avoid CD8 T cell killing preventing infected cells to present MHC molecules to get to surface of the cells.
* CD8+ T cell target recognition interacts with cancer cells with non-specific adhesion molecules: **LFA-1/ICAM-1 interaction**. If antigen-specific recognition: stable pairing and focused release of effector molecules.
* CD8+ T cell recognition and killing of cancer cells: no need for costimulatory signal.
* Cancer cells downregulate MHC I to avoid CD8+ T cell killing.

**NK (CIA)**

* They recognize MHC I molecules. Low expression of MHC I molecules will turn off the inhibition and promote killing of target cell.
* Also have Fc receptors (receptors for the constant region of antibody) when bound induces NK cells to release cytotoxic granules: granzymes B and perforin.
* antibody-dependent cell-mediated cytotoxicity (ADCC): Fc receptors on NK cells recognize bound antibodies on surface of target cells. Cross-linking of Fc receptors activate NK cells to kill.

**MHC class I/MHC class II**

1. MHC class I molecules is loaded in the endoplasmic reticulum (ER) and expressed on all cell types and pathogens intracellular.
2. MHC class II molecules is loaded within fused vesicles where it fuses with the lysosome. Only for CD4 T-cells. Only presented on APCs. Used for B-cell activation and T-cell priming.

CD4+ T-cells help to stimulate CD8 T-cells through another co-stimulatory interaction CD40-L and pro-stimulatory cytokines: IL-2.

**Extravasation**

* Extravasation refers to the process by which cells, particularly white blood cells (leukocytes), exit the bloodstream and migrate into the surrounding tissue.
* Once at the site of infection, CD8 cells engage with cells with nonspecific adhesion molecules, if they don’t recognize antigen-specific molecules, they move on to next cell. Otherwise, activation signals are sent to CD8 cells to kill infected cells.

**Memory cells**

Central memory cells and effector memory cells.

**Vaccines**

Somatic hypermutation antibodies: during the immune response, in the lymph node, B-cells specific for specific pathogen proliferate many times. Mutations in antibiotic sequence of B-cell DNA, resulting in antibodies less functional. However, some with higher affinity antigen receptors for the pathogen. B cell class switching can occur where B-cells switch from producing IgM antibodies to producing IgG.

# Autoimmune Diseases

* **Psiorasis:** main meds are corticosteroids,
* **RA**: genetic and sex risk factors: women 3 x. Treated with disease-modifying antirhumetic drugs.
* **Grave’s disease:** women 6 x. irregular heartbeats, ocular abnormalities. 3 main therapies: anti-thyroid hormone drugs, radioactive iodine, and surgical removal.
* **Hashimoto’s disease**: hypothyroidism. Women 8 x. High cholesterol (heart disease). treatment: thyroid hormones.
* **Systemic lupus erythematosus**: mediated by antibodies nucleic acids. Systemic immunosuppression through glucocorticoids.
* **Sjogren’s syndrome** mainly affects exocrine glands. Tier substation and oral mucosa lytic agents.
* **Crohn’s disease**: chronic inflammation of bowel: diarrhea, rectal bleeding, and abdominal cramps. Corticosteroids and diet.
* **Multiple sclerosis**: common in developing countries and immune response to the myelin sheath of the neurons. Sensory disturbances in the limbs, prickling or tingling, numbness, itching, muscle loss control. Reduce symptoms: steroids.
* **Type 1 diabetes**: Scandinavia and North America. Destruction of pancreatic beta cells leading to hyperglycemia. Insulin: glycemia control.

# Distinguishing Foreign from Self

**T cell tolerance**

* Central T cell tolerance occurs in the thymus.
* Takes place wen Tcells leave the bone marrow and end in the thymus. T cell receptor developed through genetic recombination: **VDJ recombination.** T-cells when they come into the thymus they will be selected as CD4 and CD8 T-cell with corresp. TCR. T-cells developed into these cells and not self-reactive, dependent on antigen binding.
* T-cells not recognizing self MHC peptides, negative selection, die**: death by neglect**. Those which bind too strongly are also eliminated to remove self-reactive T-cells, **positive selection**.
* **Auto-immune regulator Expression (AIRE):** transcription factor. Expressed in the medullary thymic epithelial cells that act as APC. Turns on peripheral genes in thymus, enforces negative selection and plays a role in the secondary lymphoid organs, further eliminating auto-reactive immune cells. Express insulins to eliminate self-reactive cells.

**B cell tolerance**

* Receptor rearrangement occurs in bone marrow.
* B cells can be rescued and can create a new B cell receptor through receptor editing. Soluble antigen and binding to T-cell can make B-cells to become anergic.
* Somatic hypermutation will change their affinity for their antibody or BCR.
* Antigen segregation: there are certain sites in the body which are immunologically different, **immunologically privileged sites**, more highly regulated.

**Peripheral tolerance**

* Lack of infection results in lack of dangerous signal or co-stimulatory signal. These self-antigen cells without co-stimulation will be deleted or become **anergic**. Low self-antigen levels lead to **ignorance**.
* **Phenotypic skewing:** to become immune regulatory.

**Regulatory tolerance**

* TGF-beta: T-cell becomes Treg.
* IL-10 and TGF-beta inhibit other self-reactive T cell.

**Tregs**

* From thymus or inducible in periphery.
* TRAIL ligand induces death.

# When Tolerance fails

* APECED
* Defective mutations in AIRE gene which provides an autoantigen screening in thymus to eliminate strong autoantigens in reactive T-cells.
* Trauma can induce autoimmune response: whence cells die to trauma, they release DAMPs (heat shocks, HMGB1) which activates DCs.
* Damp: nucleic acids. Sensing od DNA occurs both through endosomal sensors and cytosolic sensors.
* Type 1 Diabetes: CTLA4 acts as a negative co-stimulatory signal when it is present, it inhibits activation response. When loss of function, attack of pancreatic beta cells. IL2R (activation of T-cells and Treg), PTPN22 and insulin (antigen of the attach on beta cells).
* Defects in IgM antibodies production or C3b or C1q lead to dysregulation of removing apoptic cells.
* **Molecular mimicry**
* An antigen from a pathogen elicits a response looking like an autoantigen: **cross-reactivity**.

# Allergy Prevalence

* Two phases: immediate and late response.
* Anaphylaxis: systemic response can lead to drop in blood pressure, vomiting, swollenness.
* IgE quantification

# Allergic Immune Response

Mast cell secretory granules can cause strong pro-inflammatory response.

To release these granules, mast cells need to bind to specific IgE antibodies through Fc epsilon receptors on their surface. Then when bound, they need to bind antigen to be clustered.

* Special activation to make B cell to switch their antibody type to IgE (instead of IgG)
* Mast cells provide CD40L and IL-4 to B cells to stimulate IgE production.
* **Th1 helps fight intracellular bacteria (IFN-gamma)**
* **Th2 cells activate eosinophils, mast and plasma cells (IL-4, 5 and 13)**
* **Th17 cells helps activate neutrophils to fight extracellular pathogens.**
* **Tfh cells activate B-cells to promote class switching to fight extracellular pathogens.**
* **Tregs**.
* **Allograft recognition**
  + **Direct****alloantigen recognition:**

Donor APCs migrate to secondary lymphoid tissue and stimulate alloreactive T-cells. T cells have receptor specific for:

Self MHC - foreign peptide

Allogeneic MHC molecule whose structure resembles self-MHC-foreign peptide complex or a structure formed by both allogeneic MHC molecule and bound peptide.

MHC is from donor.

* **Indirect alloantigen recognition**:

Uptake of allergic proteins by recipient own APCs, and presentation to T-cells by self-MHC molecules. Recipient professional APC takes up allogenic MHC molecules and minor histocompatibility antigens present in the graft, presents processed peptide of allogeneic MHC molecule bound to self MHC molecule.

**Alloantibody**: antibody produced against non-self-antigens from another member of the same species

* **Viruses**: DC => Il-12 -> NK cells to produce IGN-gamma, naïve CD4 T-cells: Th1 cells
* **Worms**: DC => NK cells to produce IL4 => Th2
* Cutaneous exposure route causes basophil recruitment, DC, Th2
* Oral exposure: vitamin A, DC, Treg, suppression of Th2
* **Basophils**: rare, express Fc epsilon receptors, unlike mast cells, responsible for initiating and maintaining Th2 responses rather than mediating symptoms
* **Eosinophils**:rare, associated with Th2 responses, like basophils, recruited to allergic zones, contains secretory granules to destroy parasitic infections.
* C5 upregulates Fc receptors of mast cells to induce their degranulation.
* Type I reaction: IgE, mast cell activation.
* Type II and III: IgG
* Type IV: Th1 or Th2 cells
* High dose of shot: can lead to anaphylactic shock.

# Causes and Rise of allergies

**Celiac disease**

Association with HLA-DQ2, CTLA-4, trans glutaminase (tTG)

Initiated by damage to the intestine

Antigen sensitization => IgG

# Tumor antigens

Cancer immunoediting

* **Elimination, Equilibrium**: tumor cells mutate to not express antigens recognized by immune system and **Escape**: proliferation.
* CAR: T-cells taken from the body, expanded, and reprogrammed so that they can have a receptor against a cancer antigen, reinjected back.
* Checkpoint inhibitors: turn immune cells back on.
* Normal cells: self-peptides bound to MHC molecules and presented: no T-cell receptors that recognize that peptide MHC complex.
* If a point mutation occurs in a self-protein in a cancer cell, a new peptide could be created and a TCR could be receptive to that new MHC peptide combination.
* Point mutation occurs and new epitope (specific piece of antigen recognized by T-cell) is created for T-cell recognition.
* Differentiated antigens only express in the tissue from which they arose.
* DC need to mature to present antigen to T-cells. Immature DCs: patrol the tissues, antigen uptake, low surface MHC II, high intracellular MHC II, high FcR, low CD40, 80, 86 and IL-12. DAMPs => antigen presentation, high surface MHC, low FcR, high CD40, 80, 86, IL-12 (co-stimulatory T-cell activation).
* Signal 1 (activation): MHC loaded with antigen binds TCR, signal 2 (survival vs no signal 2 like with MHC with self-peptide and T-cell is not surviving): costimulatory signal DC is CD28 on T cell binding to B7.1 B7.2 on the APC, signal 3 (differentiation): cytokines IL-6, IL-12, TGF-beta
* Immunological synapse: interface between T cell and APC. To interact clustering of signaling and adhesion molecules: signaling molecules the central supramolecular activation cluster or **cSMAC**, adhesion molecules in the pSMAC: LFA1:ICAM-1.

**Mechanisms of Immune Evasion**

* Low immunogenicity
* Tumor treated as self-antigen
* Antigenic modulation: tumor cells selectively lose or downregulate antigens targeted by the immune system.
* Tumor-induced immune suppression
* Tumor-induced privileged site
* PD-1 pathway suppresses T cell activity.

# Inflammation and Wound Healing

**Phases of wound healing**

Inflammatory, Proliferative, Maturation

**Acute inflammation**

Platelets at the site of the wound, release PDGF, and other chemoattractant which cause neutrophils to extravasate from the bloodstream.

**Chronic inflammation**

Macrophages and T-cells will similarly extravasate. These cells produce even more cytokines like **CXCL8**, which recruit T-cells to the site, and chemokines the cells required for the proliferation phase.

**Leukocyte Extravasation**

Rolling, tight binding, diapedesis and migration. Integrin molecules: lower to higher affinity state.

# Protein adsorption

VRoman effect: effect by which the proteins are adsorbed on the material surface and replace each other over time, this can lead to higher affinity proteins: IgG and complement proteins.

**Classical pathway**

C1q binds to IgG molecules. C1q => cleaves C4 => C4a and (C4b) => cleaves C2 => C2a and C2b => complex C4b,C2b => is an active C3 convertase

**Alternative pathway**: C3 or C3b. Hydrolysis of C3b binds to factor B, factor B cleaved by factor D

All pathways generate a C3 convertase, which cleaves C3, cleavage of C3 exposes the thioester bond. C3 then can be attacked by water molecule: soluble C3b or by an hydroxyl or amin group on surface of biomaterial leaving C3b bound to the biomaterial and releasing C3a. =>

* C3a and C5a recruit phagocytic cells and promote inflammation
* Phagocytes with C3b receptor engulf and destroy pathogen/biomaterial

Amplification of classical pathway: C3b binds to factor B, factor B cleaved by factor D into Ba and Bb: C3bBb is a C3 convertase.

Proteins adsorption can increase opsonization of the biomaterial which can be phagocytosed.

**On host cells**

Existence of complement-regulatory proteins CR1, H, MCP and DAF bind to C3b and will displace BB so C3 convertase can’t be formed.

* Monocytes
* LPS/IFNgamma => M1 => IL-1, TNFalpha, IL-12, CXCL12
* IL-4,13,10 => M2. M2 macrophages produce IL-10 and TGF-beta
* Foreign body giant formation is induced by IL-4 and IL-13
* T cell activation: CD28. CD28 in cSMAC.
* Tregs is a sink for IL-2 (instead of CD8: differentiation and proliferation)
* CD4: MHC II
* MHC: to activate T-cells
* High: Deletion and conversion to Treg
* Medium: maturation of T-cells
* Low: Deletion of T-cells
* Neutrophils: attach to biomaterials via receptors for IgG and C3b
* Alternative pathway triggered by C3b hydrolysis
* C3b adsorption to biomaterials results in cell adhesion
* A tumor might secrete IL-10 and TGF-beta, IDO
* Intracellular pathogens: MHC1 to CD8 TCRs