Summary for mid material immunology

THIS APPLIES FOR ALL SECTIONS

Heyam Awad

Innate immune system

Innate immunity is composed of:

- 1. Epithelial barriers: skin, respiratory and gastrointestinal epithelium.. These prevent entry of microbes
- 2. Phagocytic leukocytes: macrophages and neutrophils
- 3. Natural killer cells
- 4. Complement system proteins

Adaptive immune system

- Composed of lymphocytes (B and T lymphocytes) and their products
- Each B or T cell responds to a specific antigen. B cells recognize antigens by B cell receptors which are antibodies .T cell recognize antigens by T cell receptors

Antigen

- Antigen is a molecule that can induce an immune response. It can be peptides, polysaccharides or lipids. They have to be foreign to the body (non-self)

B and T cell Receptor diversity

- Within the chromosomes encoding heavy chains there are multiple copies of DNA molecule (gene segments) called V,D,J,C segments. Each of these segments has several copies and each copy is slightly different : for example there are 40 different V , 25 different D and so on.
- B and T cells mix and match these segments to create the desired diversity. This process is called modular design .This is similar to creating countless numbers of proteins by mixing and matching 20 amino acids ,or to creating countless words by mixing the letters of the alphabet

Complement system

- The complement system is composed of more than 20 proteins synthesized in the liver.
- They circulate in the blood in an inactive form.
- To perform their function these proteins must be activated by proteolytic cleavage.
- Each complement component is cleaved, when stimulated, by an enzyme (a convertase) that cleaves the complement components to two parts, a smaller part named a (like C3a for example) that is soluble and performs certain functions like acting as a chemotactic agent ,and a larger component termed b (C3b for example) which can be fixed to tissue.

Complement system

- Composed of 20 proteins synthesized in the liver
- These circulate in the blood in an inactive form.
- Activated when needed by three ways:

1. Classical pathway

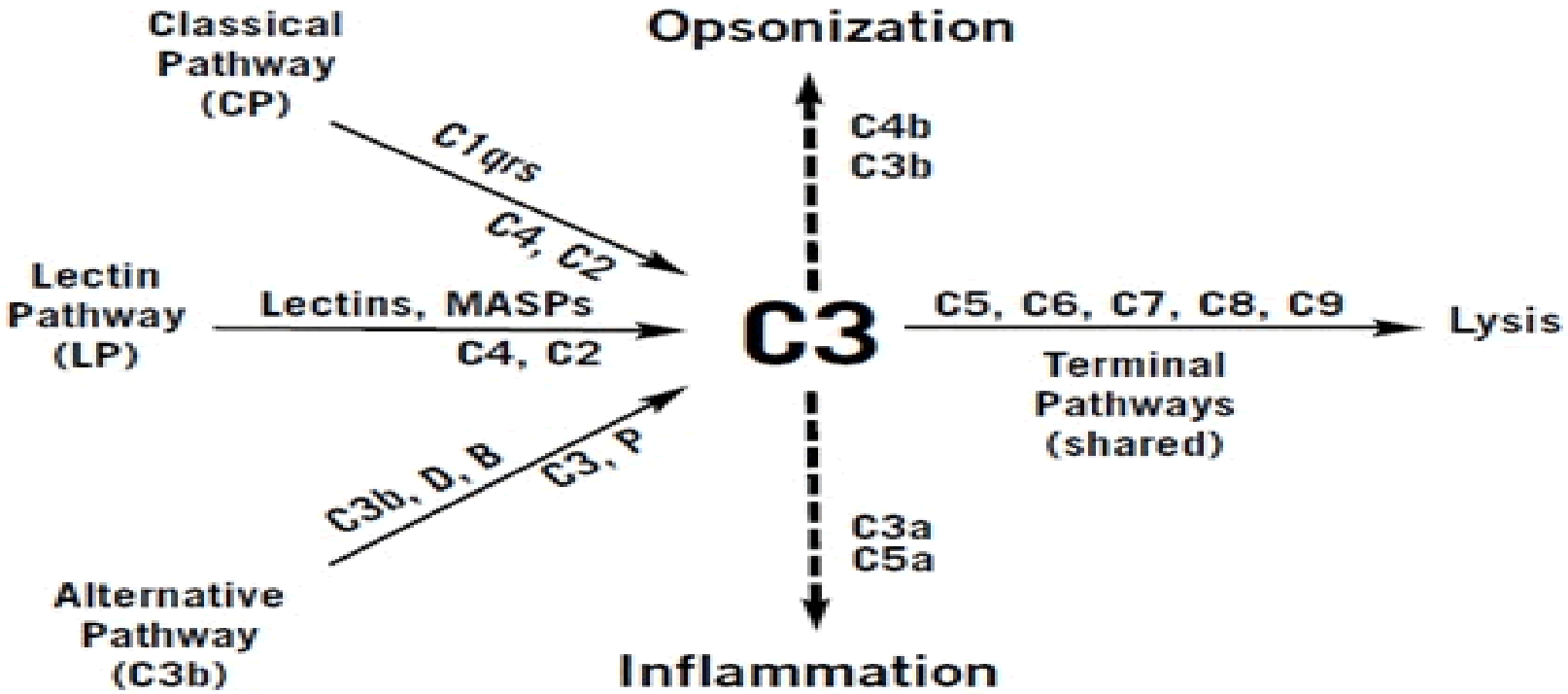
2.Alternative pathway

3.Lectin mannose pathway

- All three pathways cause activation of C3 convertase which plays a central role in complement effects.

- Once activated (by any of the three pathways) bacterial killing happens through three mechanisms….will be mentioned in a minute

complement


Classical
Pathway
(CP)
C1qrs
C4, C2
Lectin
Pathway
(LP)
Lectins, MASPs
C4, C2
Alternative
Pathway
(C3b)
C3b, D, B
C3, P
Opsonization
C4b
C3b
C3
C5, C6, C7, C8, C9
Terminal
Pathways
(shared)
Lysis
C3a
C5a
Inflammation

Alternative pathway

- C3 is the most abundant complement protein.
- C3 is **continuously cleaved to c3b (spontaneous cleavage)**
- C3b is very reactive and can bind to amino or hydroxyl groups
- On bacterial surface these 2 groups (amino and hydroxyl) are abundant
- If no bacteria and nothing to react with c3b neutralized within 60 microseconds by binding to water

- If C3b finds a hydroxyl or carboxyl group on a pathogen it is stabilized and another protein(protein B)attaches to it.
- Protein B is cleaved by protein C to create protein **Bb**
- Now the C3b and the Bb form C3bBb = C3 convertase.
- The C3 convertase cleaves more and more of C3.
- A chain of reaction follows, where complement proteins are activated by cleavage done by the convertase enzymes
- C6- C9 form the membrane attack complex (MAC) which causes lysis of the pathogen,

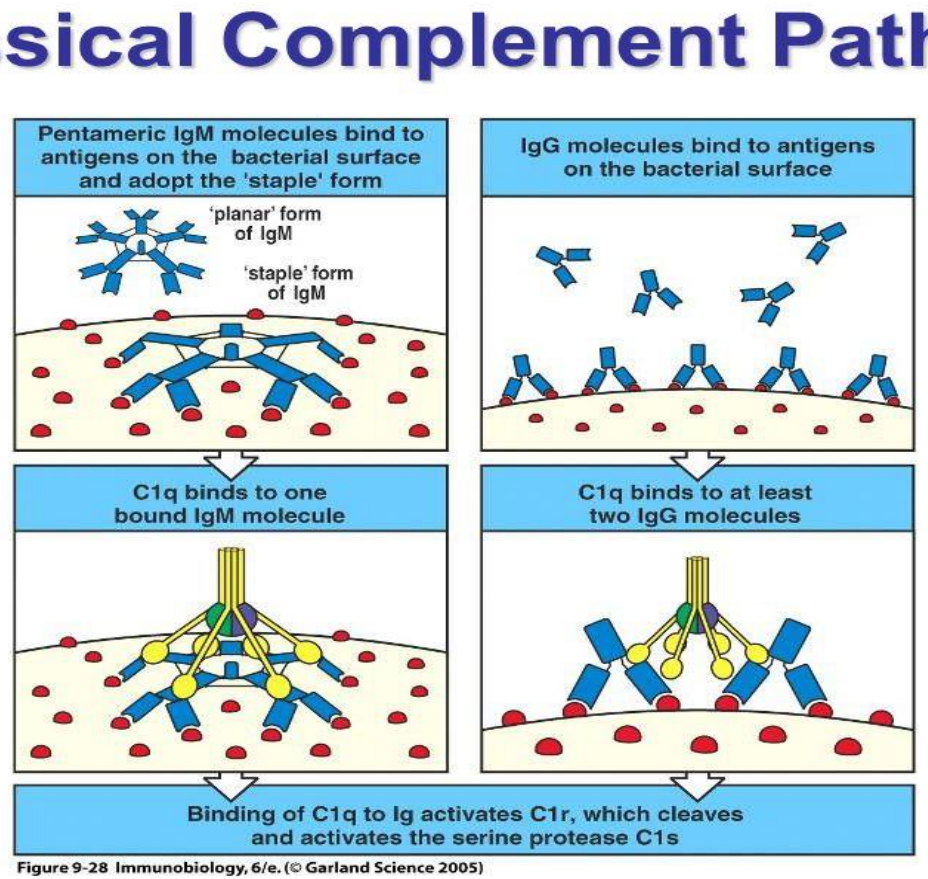
Why complement system doesn't attack our tissues

- C3b can be inactivated by MCP enzyme
- DAF= decay accelerating factor can accelerate destruction of C3 convertase
- CD59 prevents C9 to assemble and form MAC

Classical pathway = activation via antigen-antibody complexes

- first step is activation of the **C1-complex**.
- The C1-complex is composed of 1 molecule of C1q, 2 molecules of C1r and 2 molecules of C1s, or ***C1qr^2s^2*.**
- The activation occurs when C1q binds to **IgM or IgG** complexed with antigen.
- Such binding leads to conformational changes in the C1q molecule, which leads to the activation of two C1r molecules.
- C1r is a serine protease. They then cleave C1s (another serine protease). The $C1r^2s^2$ component now splits C4and then C2, producing C4a, C4b, C2a, and C2b.
- C4b and C2b bind to form the classical pathway C3-convertase (C4b2b complex) (or C4b2a) both are correct, don't worry, which promotes cleavage of C3 into C3a and C3b.

This shows the C1 complex activation.. Note that the immunoglobulin leading to the activation is either IgM or IgG



Lectin activation pathway

- Mannose binding lectin (MBL) is a protein produced from liver and is present in moderate amounts in blood and tissues.
- Lectin = means a protein that can bind a carbohydrate molecule.
- Mannose: is a carbohydrate found n the surface of many pathogens
- MBL binds to carbohydrates found on many pathogens but it doesn't react with carbohydrates on human tissues.

- MBL in the blood binds to another protein called MASP
- **MASP can directly cleave C3 and act as a c3 convertase but it also stimulates the production of C4b2a which acts as the main c3 convertase in this pathway.**
- So when MBL recognizes a pathogen's mannose it binds to it and the MASP is activated.

How complement system kills pathogens

- 1. by the MAC causing lysis of cells. Note: MAC is a complex of several complements, C9 is the most important, this complex attacks membranes of pathogens by creating holes within them.. This allows water to enter the cell.. Lysis happens
- 2. C3b acts as an opsonin (an antibody or other substance that binds to foreign microorganisms or cells, making them more susceptible to phagocytosis) .. It coats the pathogen and targets it for phagocytosis
- 3. C3a and C5a are anaphyltoxins that elicit an inflammatory response and act as chemotactic agents

macrophages

- Macrophages originate from bone marrow precursors.
- They circulate in the blood as monocytes
- Monocytes in tissues are called macrophages

Macrophages have three phases or levels of activation

- Resting
- Activated = primed
- hyper-activated

Resting macrophages

- Resting macrophages are present beneath the skin and body surfaces , they get rid of debris and of dying cells
- These express few MHC II
- They just collect garbage

Activated macrophages

- Activated macrophages = primed
- Activated by several factors mainly interferon gamma produced from T helper and NK cells
- Activation happens in response to pathogens
- Activated macrophages upregulate MHC II producing loads of it
- By increasing their MHC II they can present antigens to T helper cells.. They act as antigen presenting cells

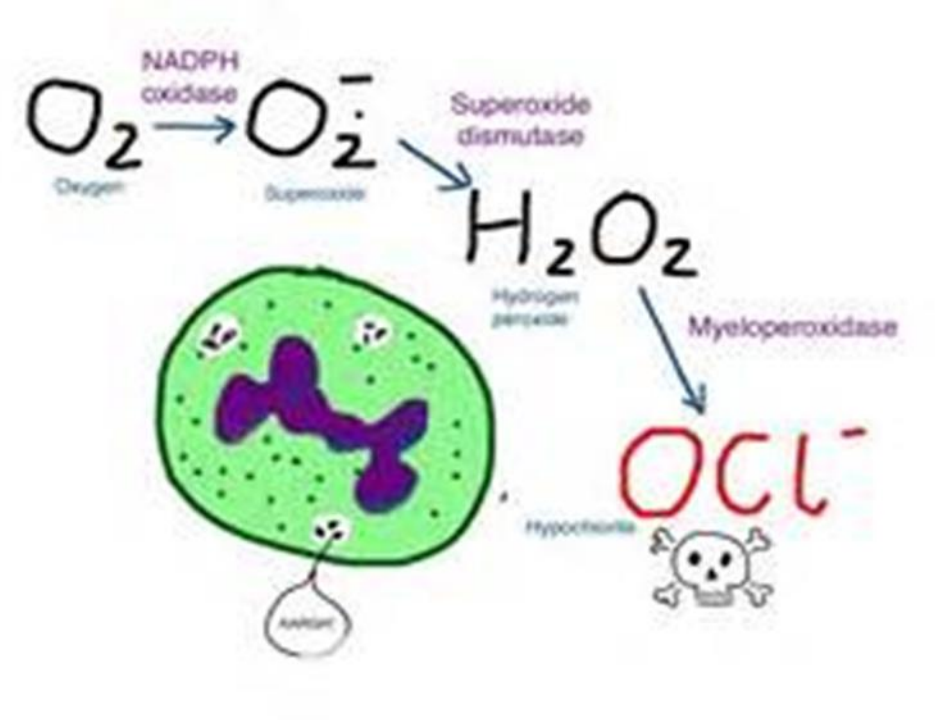
Hyper activated macrophages

- Hyper activated when receive a direct signal from an invader
- The signal is usually a lipopolysaccharide or a mannose
- When thy are hyper activated they stop proliferating
- Instead they become large and increase their phagocytic activity
- Also they produce TNF (tumor necrosis factor)..
- TNF Kills tumor cells and pathogens and also activates other immune cells

phagocytosis

By

- lysosomal enzymes
- Oxygen radicals
- Nitrogen radicals



neutrophils

- Are phagocytic.
- Note: neutrophils do not act as antigen presenting cells
- Originate from bone marrow precursors
- Circulate in the blood.
- Life span 5 days.

Recognition of microbes

Phagocytes recognize microbes by: Pattern recognition receptors, two types

- Pathogen associated molecular patterns = toll like receptors
- Damage associated molecular patterns = inflammasomes

Toll like receptors

- Microbial sensors.
- 10 mammalian types:
- Can recognize bacterial products : endotoxins, lipopolysaccharides or DNA.
- Can recognize viral products: RNA

- They recognize a pattern: e:g liposaccharides in general, not a specific type, DNA chains not specific sequences.

Natural killer cells

- Mature in the bone marrow
- Half life one week
- Manly in blood ,spleen ,liver
- Can produce interferons and other cytokines
- Activated by: interferon alpha and beta+ lipopolysaccharide
- NK kill cells by apoptosis.. Mainly through Fas –fas ligand

NK receptors

- Have 2 types of receptors activating and inhibitory receptors
- Inhibitory recognizes MHC 1 on cells
- Activating binds to altered protein on the target cell
- Altered due to viral infection or cancer
- Balance between activating and inhibitory signals determines if the NK will kill the target cell or not.

- Note: NK cells are lymphocytes, but they do not express B nor T cell receptors.

NK receptors

1. Inhibitory receptors (killer inhibitory receptors = KIR) . These recognize MHC 1 on cells. Once these receptors are engaged they will activate phosphatases PTP which will inhibit killing via dephosphorylation.

Within the cytoplasmic portion of the receptor there are amino acid sites that bind tyrosine kinase and are inhibited by dephosphorylation. These amino acid sites are called ITIMS =(immunoreceptor-based tyrosine inhibiting motif). Which simply means that once engaged these receptors have regions (motifs) that are inhibited by tyrosine kinase binding.

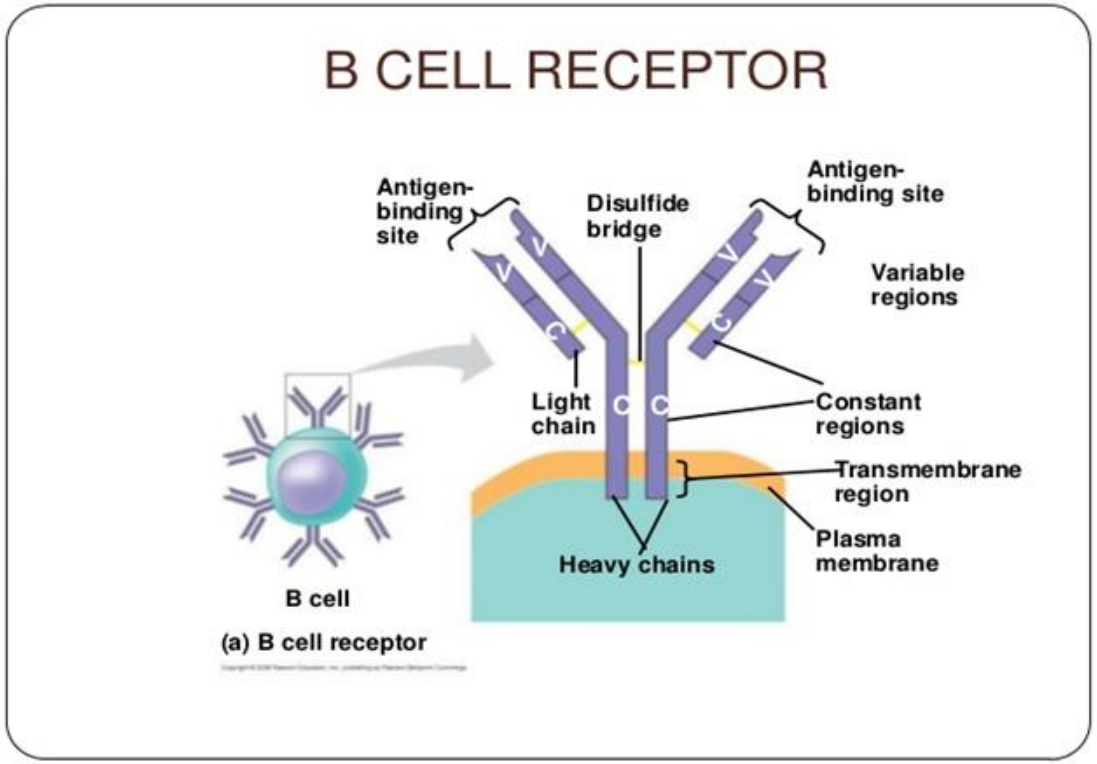
2. Activating receptor: binds to altered protein on the target cell (viral infection or cancer). Once NK cells recognize their target they send signals which will activate protein tyrosine kinase PTK and kill the cell. These contain ITAMS = immunoreceptor based tyrosine activating motifs.

- NOTE THAT the balance between activating and inhibitory signals determines if the NK will kill the target cell or not.

B cells

- The specific immune system is composed of the B and the T lymphocytes. B cells produce antibodies (AB), also called immunoglobulins (Ig) which circulate in the blood, and this is called humoral immunity (humoral means something related to body fluids, and AB circulate in a body fluid, the blood), whereas T cells are responsible for cellular immunity

B cell receptors



NOTE

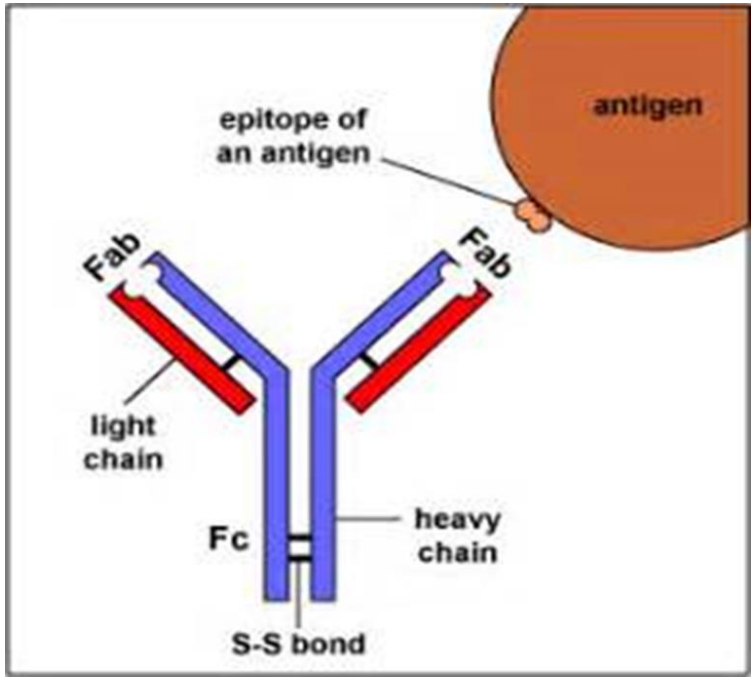
- BCR is an immunoglobulin (Ig).
- Note that the receptor has a *transmembrane* region, which is a hydrophobic amino acid sequence that anchors the BCR to the cell membrane.
- This transmembrane sequence is not present in the circulating immunoglobulins and that’s why Ig do not bind to the membrane, but circulate in blood instead

- Each B cell has one unique BCR that is specific for one antigen. This receptor is selected through **modular design** during B cell maturation in the bone marrow
- Genes coding for **both** heavy chain and light chain are assembled through modular design.
- Genes for BCR heavy chain are found on chromosome 14. Each B cell contains 2 copies of chromosome 14 (maternal and paternal). Each copy starts a rearrangement of DJ segments of the heavy chain gene and delete the DNA in between. Then a V segment is chosen and joined to the DJ , again by deleting the DNA in between
- Next to the J segment there are C segments that code for the constant region. Closest to the J are the c segments that code for IgD and IgM, so one of these is chosen for the BCR. This means that by default the BCR is of immunoglobulin class D or M, not any other!

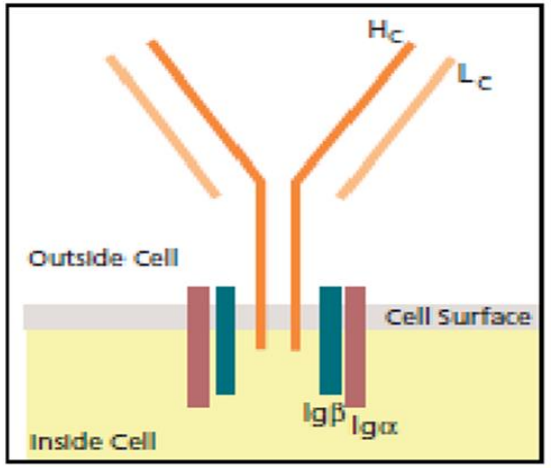
- BCR contains also a light chain, which is assembled through modular design. For the production of a BCR we need the B cell to have **productive arrangement** of the light chain but also it is must that the light and heavy chain in that cell fit together to create the BCR.
- If no productive light arrangement or if the light and heavy do not complement each other, the cell *dies by apoptosis* .

How do BCR recognize the antigen

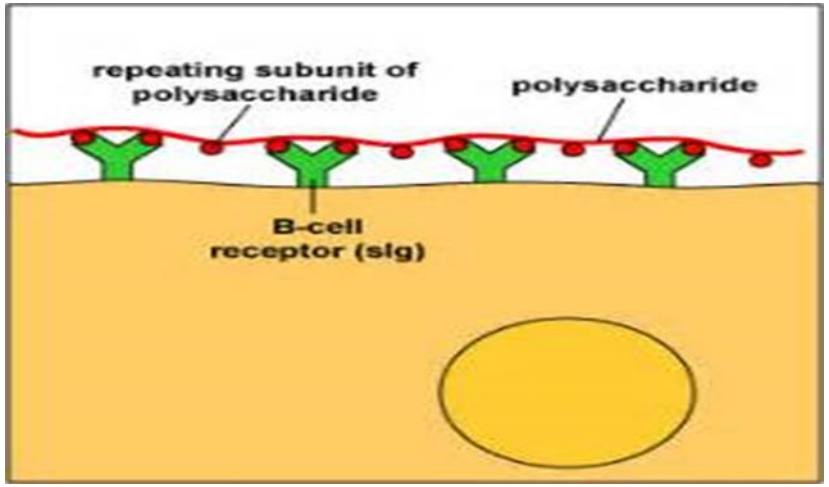
- Each BCR recognizes one specific antigen called the cognate antigen (basically this means the complementary antigen) . BCR binds to a tiny part (6-12 amino acids) of the antigen. This is called the epitope.



- Once the B cell binds to the epitope it needs to send a signal to the nucleus in order to start immunoglobulin synthesis .This signaling is done through two segments that bind the intracellular part of the heavy chain, these are called Ig alpha and Ig beta
- **Note that Ig alpha and beta do not recognize antigens. They only act as second messengers**



- For signaling to the nucleus to happen, we need **clustering** of B cell receptors. This clustering is called **cross linking**. Cross linking happens when adjacent BCR recognize repeated epitope sequences on an antigen or when the receptors recognize several epitopes from several closely located antigens on a bacterial surface



- This cross linking is important because it allows enough signals to be transmitted to the Ig alpha and beta to start the cascade of second messengers to convey a message to the nucleus.
- On B cells there is another type of receptor; a complement receptor (CR2 or CD21)that binds complement opsonins like C3b (note that when c3b acts as an opsonin sometimes it's called iC3b, so don't be confused) and C3d which is recognized by the CD21
- If a bacterium is opsonized the B cell will recognize two things in the bacteria: the BCR (AB) recognizes the epitopes and the complement receptor will recognize the complement component (the opsonin)
- If both receptors act together the need for crosslinking is decreased and the second messages are amplified.

- Note: the complement receptor is considered a co-receptor, because of its effect in helping the BCR to be stimulated and in decreasing the need for crosslinking

Co stimulation

- Once the antibody recognizes its cognate antigen, the B cell can be activated. There are two ways of activating B cells: 1. **T cell dependent** and 2. **T cell independent activation**.
- B cell activation ***always*** needs two signals, the **First signal is the BCR crosslinking.** The second signal is called a **co-stimulatory signal**, which can be achieved through T cell dependent or T cell independent activation.

- In **T cell dependent** activation, the second signal occurs when a ligand on the surface of T helper (Th) cell is attached to its complementary molecule on the surface of a B cell. These molecules that produce the costimulatory signal are CD40 on the B cell and CD40L (standing for CD40 ligand) on the T cell
- **T cell independent** activation occurs if the antigen has many repeated similar epitopes. This will cause clustering (cross linking) of many BCR. Bacterial carbohydrates usually have these repeated epitopes. However, another signal is still needed, which is a danger signal usually recognized by toll like receptor

- T cell independent activation gives B cells a chance to be activated quickly without waiting for T cells to be activated.
- This is important in: most B some bacterial infections. For example cells that are activated in the T cell independent activation are present in the spleen. These act quickly against Streptococcus pneumonia infection. People who have their spleen removed are at risk of having clinically significant streptococcus pneumonia infection.

T cell dependent activation of B cells

- If B cells are activated via this mechanism, T helper must be activated first through recognizing its cognate antigen presented by an antigen presenting cell.
- Then B cell is activated and proliferates .
- After that B cells can differentiate to plasma cells and produce immunoglobulins

- Cross linking of BCR and subsequent activation of the B cell stimulates kinases (Src family kinase, the kinase causes phosphorylation of the ITAM tyrosines of Igα and Igβ, followed by the recruitment and activation of other kinases.
- ITAM= immunoreceptor tyrosine-based activation motifs ,these contain tyrosine residues that become phosphorylated by cytoplasmic kinases after binding of ligands to the receptors. Other protein kinases are recruited to the modified ITAMs and become activated, and these kinases contribute to further signaling by phosphorylating additional proteins,
- ITAMs also found in the cytoplasmic tails of several signaling receptors in the imune system, including the antigen receptor complexes of T and B cells as well as in NK signaling.

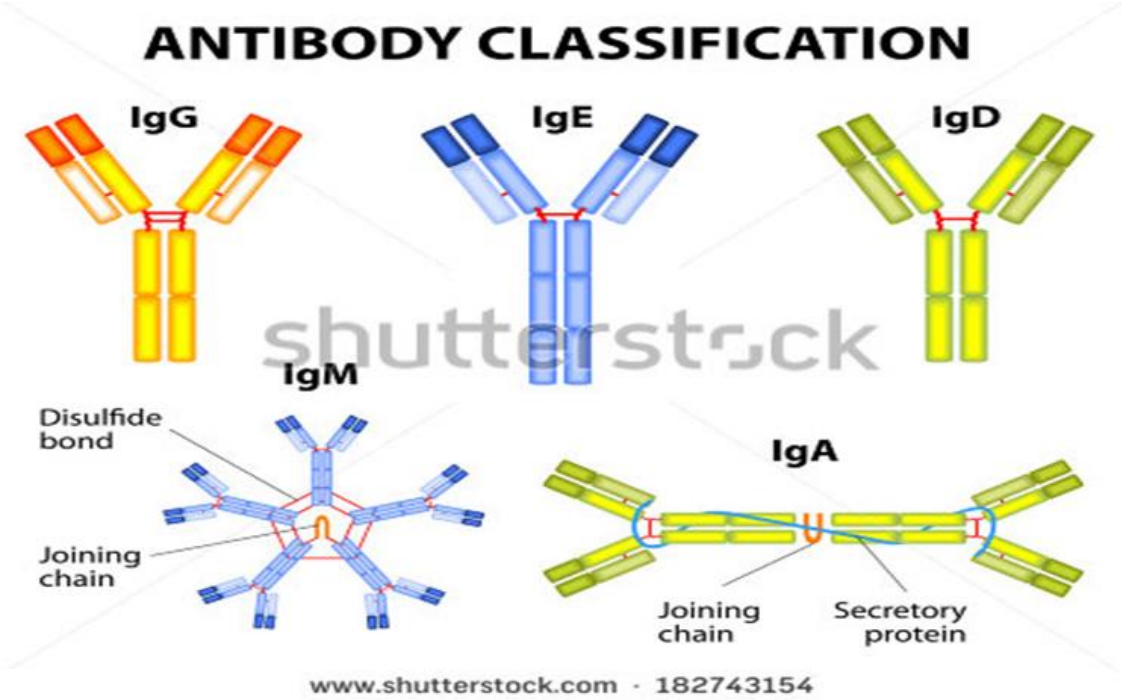
B cell maturation

- Once B cells are activated, they mature. This maturation involves three things
- 1. **AB class switching,** remember that the first AB (the default Ab produced by any B cell is IgD or IgM) but once B cells mature they can change the AB class they produce to the class suitable for the specific situation.
- 2. **somatic hyper-maturation or affinity maturation**, this is a process through which B cells can increase affinity of their immunoglobulins to antigens
- 3. **differentiation**, B cells decide if they will become plasma cells or memory cells.

AB class switching

- the C region determines the classes of the antigen because it codes for the Fc region of the AB .
- To switch antibody class the B cell changes gene arrangement of the C module.
- So with class switching only the Fc region changes without changing the variable region which means antigen recognition is not changed

AB types


ANTIBODY CLASSIFICATION
IgG
IgE
IgD
IgM
Disulfide bond
Joining chain
IgA
Joining chain
Secretory protein
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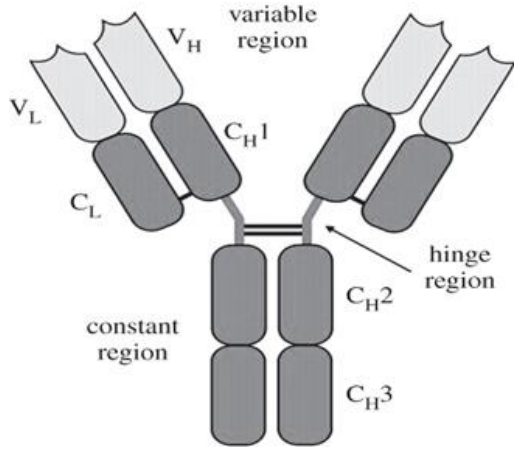
- All antibodies have a common symmetric structure composed of two identical covalently linked heavy chains and two identical light chains, each linked to one of the heavy chains.
- Each chain consists of two or more independently folded Ig domains of about 110 amino acids containing conserved sequences and intrachain disulfide bonds.
- Each domain contains 2 layers of beta pleated sheet, each layer is composed of 3-5 strands of polypeptide chains

- The N-terminal domains of heavy and light chains form the variable regions (V regions) of antibody molecules, which differ among antibodies of different specificities.
- The V regions of heavy and light chains each contain three separate hypervariable regions of about 10 amino acids that are spatially assembled to form the antigen-combining site of the antibody molecule (the binding site, called the fab region) .
- These hypervariable regions are also called complementarity- determining regions (CDR), the three hypervariable regions are called CDR1, CDR2, CDR3 and CDR3 is the most variable and has the most genetic diversity. Note that there are 3 CDR regions for the heavy chain and three for the light chain.

Hinge region

- The hinge region is a short sequence of amino acids that lies between CH1 and CH2 regions. This gives the AB flexibility so AB can bind an antigen at more than one binding site. The overall binding of all sites is the avidity.



AB half lives

- Each AB class has a different half life , half life is the average time at which the number of AB is reduced by half. IgE has the shortest half life 2 days. IgG has the longest half life of 21-28 days
- The long half life of IgG is due to its ability to bind to a receptor called neonatal fc rector FcRn which is also involved in the transport of IgG across the placenta and to fetal blood. Binding to this receptor sequesters IgG away from lysosomal degradation giving it this long half life

- Antibodies eliminate microbes by several mechanisms:
- 1. Neutralization of microbes and their toxic products
- 2. Activation of complement (classical pathway)
- 3. opsonization of pathogens to enhance phagocytosis
- 4. Antibody mediated cell dependent cytotoxicity (ADCC).. details later
- 5. Antibody mediated mast cell activation fight parazites

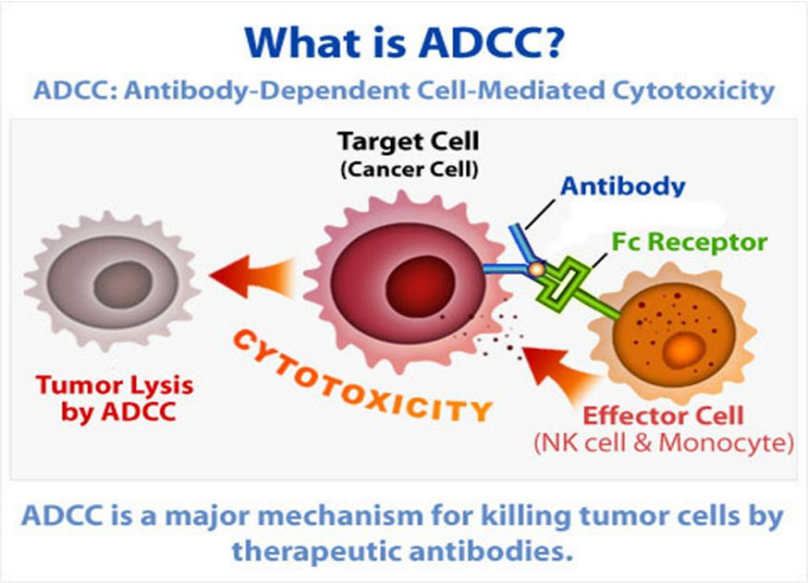
IgM

- IgM is the first AB type produced during infections. IgM is a pentamer.
- Note that complement activation through this pathway is specific to the antigen recognized by the IgM.

IgG

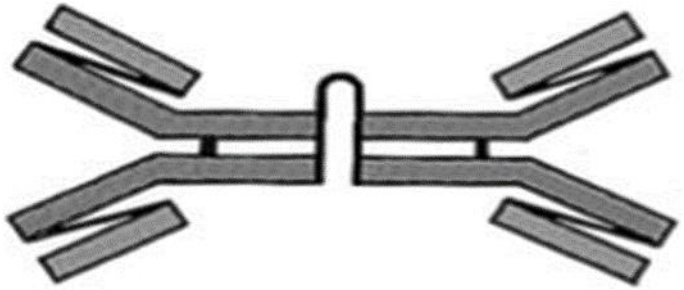
- There are different subclasses of IgG (IgG1, 2, 3 and 4) that have slightly different Fc regions.
- Each subclass has a different function. IgG1 acts as an opsonin. IgG3 fixes complement . IgG can neutralize viruses and it can pass through the placenta which gives protection to the infant
- Also because they are long lived they can be used to protect from infections like hepatitis A if one is exposed to the infection

- IgG3 also can form a bridge between bacteria and NK cells. It binds the bacterial antigen via the Fab region and the NK by the Fc region which binds to a receptor on NK. This brings the bacteria close to the NK but also stimulates the NK to kill! This is called ADCC = antibody dependent cellular cytotoxicity



IgA

- **IgG is the most abundant AB in blood… But IgA is the most abundant in the body!!**
- IgA is the AB that protects mucosal surfaces. IgA molecule looks like 2 IgG clipped together.
- This clip is important for the IgA function: it allows its movement through intestinal wall and protects it from the intestinal acidity and enzymes.

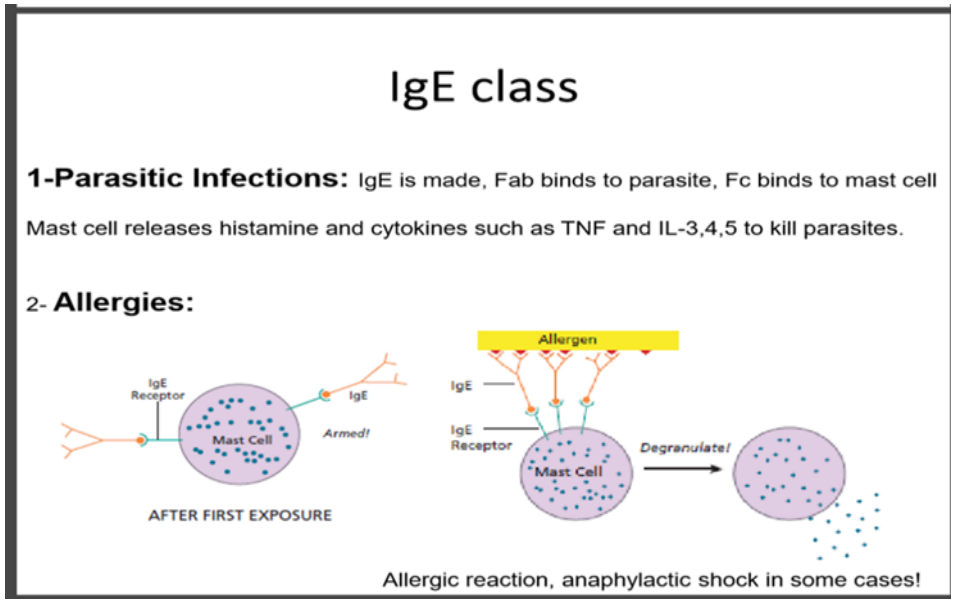


IgA

IgA

- In the intestinal lumen IgA coat the invading pathogen preventing it from attacking the intestinal cells.
- Because IgA has four Fab regions it can attach to bacteria and produce a large enough particle to pass through mucus or stool
- IgA is secreted in mothers' milk and taken by the baby during breast feeding, they coat the baby's intestine and protect it
- IgA cannot fix complement, which is good because if they do our mucosal membranes will be always attacked by complement in response to bacteria that is always there in the mucosal membranes

- IgE: Synthesized against allergens. It can coat mast cells and it defend against parasites.



Ab Classes and functions

ANTIBODY CLASS	ANTIBODY PROPERTIES
IgM	Great complement fixer Good opsonizer First antibody made
IgA	Resistant to stomach acid Protects mucosal surfaces Secreted in milk
IgG	OK complement fixer Good opsonizer Helps NK cell kill (ADCC) Can cross placenta
IgE	Defends against parasites Causes anaphylactic shock Causes allergies

Class switching

- The aim of class switching is to use the correct AB to the specific situation. So at first encounter with a pathogen IgM is always the first Ig secreted.. But according to the type and site of infection, switching occurs.
- If infection of mucosal membrane : IgA produced (switching to IgA)
- If parasitic infection: IgE
- AB switching is **controlled by the cytokines** encountered by B cells
- IL4 and IL5 : switching to IgE
- Interferon gamma causes switching to IgG3
- TGFb : IgA switch
- These cytokines are secreted by T helper which decides which ones to secrete according to the situation and the AB needed

Somatic hypermaturation= affinity maturation.

- In the VDJ region the mutation rate is high, much higher than in other cells. The increased mutation rate occurs late in the maturation of the B cell .Outcome of these mutations can be increased, decreased or unchanged affinity to antigen. Those B cells with higher affinity compete with other B cells and they predominate
- Affinity maturation: the ones with higher affinity are selected. This affinity maturation occurs in germinal centers and leads to increased affinity of antibodies during the course of a T cell–dependent humoral response. Affinity maturation is a result of somatic mutation of Ig heavy and light chain genes induced by AID (Activation induced Deaminase), followed by selective survival of the B cells that produce the high-affinity antibodies and bind to antigen displayed by follicular dendritic cells in the germinal centers. T cells also participate in selection of high-affinity B cells.

B cell differentiation: Career choice

- B cells choose to be Plasma cells or memory cells. Plasma cells are short lived, they go to spleen or bone marrow to secrete AB. Memory cells are important for quicker recognition of antigens previously encountered by B cells.
- How B cells choose to become plasma or memory? Mechanisms not understood. But **T helper cell is thought to be important in producing memory cells. T cell independent activation doesn't produce memory cells.**

T cells and importance of antigen presentation

T cells can not directly recognize antigens. The antigen must be processed and presented to T cells.

Processed means: the antigen must be cut enzymatically to suitable linear peptide chain with a certain length.

Presented means: this processed antigen binds to Major Histocompatibility molecule (MHC) and this MHC molecule with the antigen bound to it must attach to a cell membrane to be recognized to the T cell.

- Moreover, T lymphocytes recognize only peptide antigens only. And these should be **linear not conformational**.

MHC molecules

- 1. **MHC I molecules are expressed** by **all** cells. They present samples of intracellular proteins

(endogenous proteins) to cytotoxic T cells (these are also called killer T cells or CD8+ T cells)

- NOTE: do not mix killer T with natural killer, they are completely different

2. **MHC II molecules** are expressed by certain cells, mainly dendritic cells, activated macrophages and activated B cells. These process extracellular antigens and present them to T helper cells (CD 4+ cells)

- NOTE: A single T cell can recognize a specific peptide displayed by only one of the large number of different MHC molecules that exist. **This phenomenon is called MHC restriction**

Class 1 MHC

- Class 1 MHC have a binding groove that is closed at both ends, so the peptides they present must fit within the groove. So they are usually eight or nine amino acids in length . These peptides are anchored at the ends, and the slight variation in length is accommodated by letting the peptide bulge out a bit in the centre.
- These peptides must be linear to fit within the groove.
- Every human has three genes for class I MHC proteins on chromosome 6 (HLA-A, HLA-B, and HLA-C)
- note that MHC are the same as HLA = human leukocyte antigens.

MHC 1 genes

- Each MHC class1 is made of a heavy chain and a β2 microglobulin chain.
- In the human population, there are about 1500 slightly different forms of the genes that encode the three class I HLA proteins. The proteins encoded by the variants of the HLA-A, HLA-B, and HLA-C genes have roughly the same shape, but they differ by one or a few amino acids.
- So HLA molecules are polymorphic which means they molecules that have manyforms which are slightly different between individuals.
- Note: β2-microglobulin protein, it is not polymorphic

Class I MHC

- Class 1 MHC bind to **endogenous** proteins. Which means the proteins synthesized in the cell as well as proteins encoded by viruses and other parasites that may have infected the cell.
- CD 8 lymphocytes inspect the protein fragments displayed by class I MHC molecules. Consequently, almost every cell is an “open book” that can be checked by these cytotoxic T cells to determine whether it has been invaded by a virus or other parasite and should be destroyed.

MHC 1

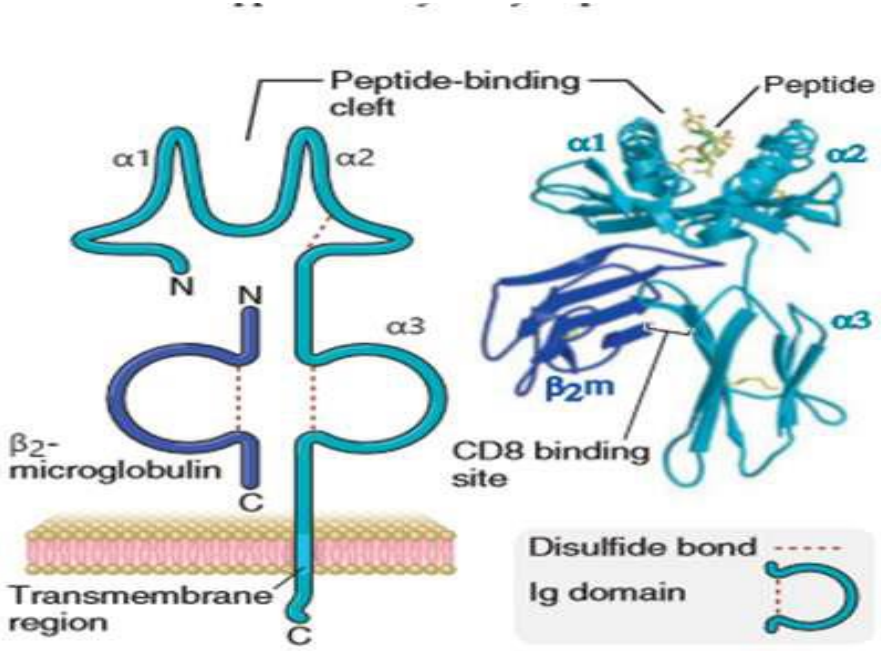


FIGURE 6.9 Structure of a class I MHC molecule. The schematic diagram *(left)* illustrates the different regions of the MHC molecule (not drawn to scale). Class I molecules are composed of a polymorphic α chain noncovalently attached to the nonpolymorphic β_2-microglobulin (β_2m). The α chain is glycosylated; carbohydrate residues are not shown. The ribbon diagram *(right)* shows the structure of the extracellular portion of the HLA-B27 molecule with a bound peptide, resolved by x-ray crystallography. *(Courtesy of Dr. P. Bjorkman, California Institute of Technology, Pasadena.)*

Class II MHC

- the class II MHC molecules are encoded by genes in the HLA-D region of chromosome 6 and are wildly polymorphic. Within the human population, there are about 700 different versions of the class II MHC molecules.
- There are three class II HLA gene loci called HLA-DP, HLA-DQ, and HLA-DR.
- Each class II MHC molecule is composed of a heterodimer of α and β polypeptides. The DP, DQ, and DR loci on each chromosome contain separate genes designated A and B, encoding the α and β chains, respectively.

- In contrast to class I MHC molecules, the binding groove of class II MHC molecules is open at both ends, so a peptide can hang out of the groove. So, **peptides that bind to class II molecules are longer than those that occupy the closed groove of class I molecules** – in the range of 13–25 amino acids.
- for class II MHC molecules, the critical amino acids that anchor the peptides are **spaced along the binding groove instead of being clustered at the ends**

Class II MHC

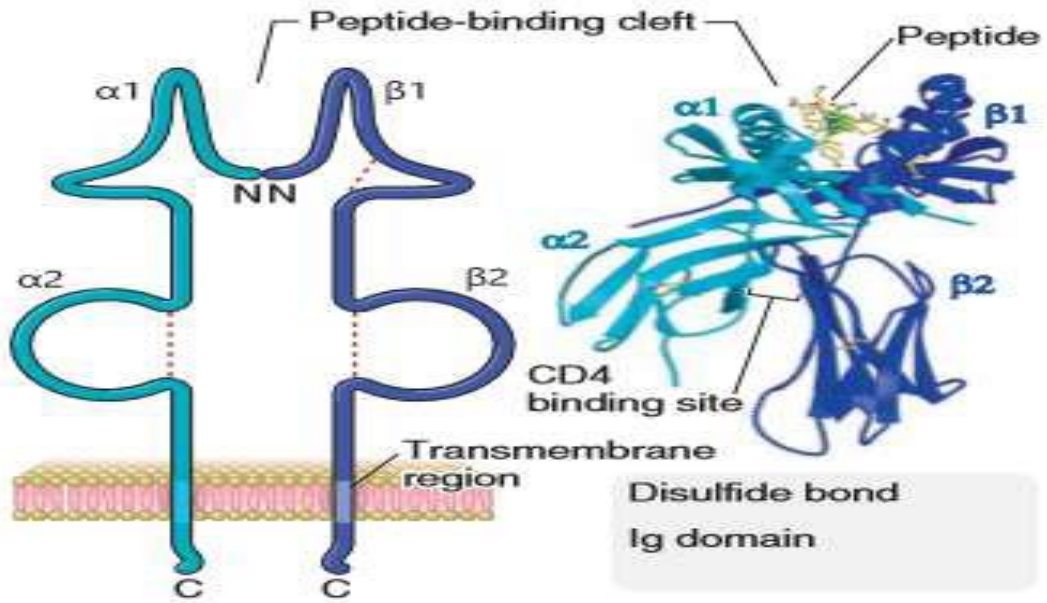


FIGURE 6.11 Structure of a class II MHC molecule. The schematic diagram *(left)* illustrates the different regions of the MHC molecule (not drawn to scale). Class II molecules are composed of a polymorphic α chain noncovalently attached to a polymorphic β chain. Both chains are glycosylated; carbohydrate residues are not shown. The ribbon diagram *(right)* shows the structure of the extracellular portion of the HLA-DR1 molecule with a bound peptide, resolved by x-ray crystallography. *(Courtesy of Dr. P. Bjorkman, California Institute of Technology, Pasadena.)*

Feature	Class I MHC	Class II MHC
Polypeptide chains	α and β_2-microglobulin	α and β
Locations of polymorphic residues	α1 and α2 domains	α1 and β1 domains
Binding site for T cell coreceptor	CD8 binds mainly to the α3 domain	CD4 binds to a pocket created by parts of α2 and β2 domains
Size of peptide-binding cleft	Accommodates peptides of 8–11 residues	Accommodates peptides of 10–30 residues or more
Nomenclature		
Human	HLA-A, HLA-B, HLA-C	HLA-DR, HLA-DQ, HLA-DP

Antigen presenting cells

- the term “antigen presenting cell” always refers to those special cells which can provide both the high levels of class II **MHC proteins and co-stimulatory** molecules required for T cell activation
- Co-stimulation usually involves a protein called B7 on the surface of an antigen presenting cell which acts with CD28 on the surface of the T cell.

There are three types of antigen presenting cells:

- 1.activated dendritic cells
- 2.activated macrophages
- 3.activated B cells.

Cell Type	Expression of: Class II Major Histocompatibility Complex	Expression of: Costimulators	Principal Function
Dendritic cells	Constitutive; increases with maturation; increased by IFN-γ and T cells (CD40L-CD40 interactions)	Constitutive; expression is increased with TLR signals, IFN-γ, CD40-CD40L interactions	Antigen presentation to naive T cells in initiation of T cell responses to protein antigens (priming)
Macrophages	Low or negative; increased by IFN-γ and T cells (CD40L-CD40 interactions)	Expression is increased by TLR signals, IFN-γ, CD40-CD40L interactions	Antigen presentation to effector $CD4^+$ T cells in effector phase of cell-mediated immune responses (T cell–enhanced killing of phagocytosed microbes)
B lymphocytes	Constitutive; increased by IL-4, antigen receptor cross-linking, and T cells (CD40L-CD40 interactions)	Expression is increased by T cells (CD40-CD40L interactions), antigen receptor cross-linking	Antigen presentation to $CD4^+$ helper T cells in humoral immune responses (helper T cell–B cell interactions)

NON-CLASSICAL MHC MOLECULES AND LIPID PRESENTATION

- Class I and class II MHC molecules are called classical MHC molecules.
- There also are non-classical MHC molecules. The best studied of these is the **CD1** family of proteins. These non-classical MHC molecules resemble class I MHC molecules in that they consist of a long, heavy chain protein which is paired with the β2-microglobulin protein.
- CD1, non-classical MHC molecules have grooves which are designed to bind **lipids**. CD1 molecules can “sample” lipids from various compartments within a cell, and can present these molecules on the surface of antigen presenting cells, where they can activate T cells.
- Obviously, CD1 presentation of lipids to T cells is an exception to the rule that T cells recognize peptides only. So far, however, it is not clear how important lipid presentation is for the immune defense.

TCR

- TCR is composed of two peptide chains, either α and β chains, or γ and δ chains.
- The genes for α, β, γ, and δ are assembled by mixing and matching gene segments, exactly like the gene rearrangement of BCR heavy and light chains (the modular design process we discussed previously).
- In fact, in B and T cells, the same proteins (RAG1 and RAG2) initiate the splicing of gene segments by making double-stranded breaks in chromosomal DNA. Please note RAG = recombination activating gene.

- When the gene segments are mixed and matched, each T cell assembles a receptor which is either an αβ or a γδ receptor, but not both.
- Over 95% of the T cells in circulation have αβ T cell receptors, these are called traditional T cells.

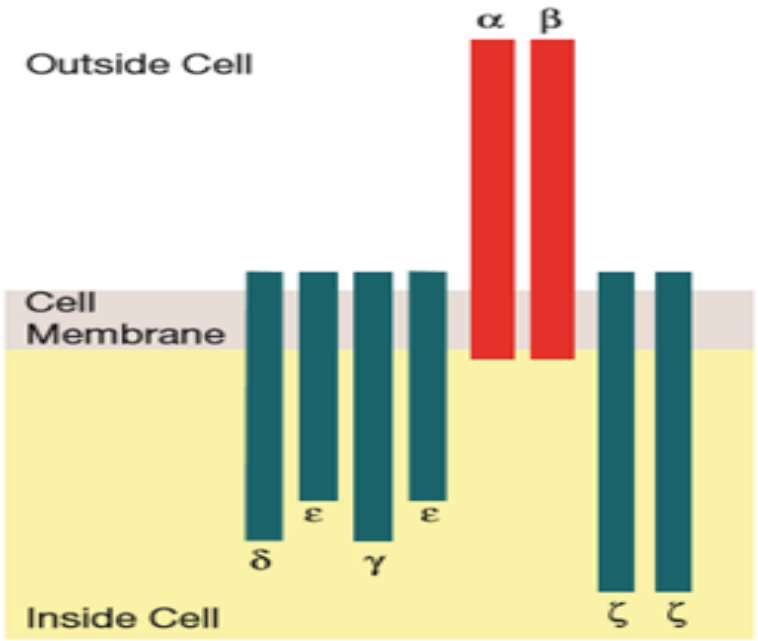
Traditional T cells

- The αβ receptors of “traditional” T cells recognize a complex composed of a peptide and an MHC molecule on the surface of a cell, and a “mature” T cell will have receptors that recognize peptides associated either with class I MHC molecules or with class II MHC molecules.
- Importantly, the αβ receptors of a traditional T cell recognize both the peptide and the MHC molecule, and unlike B cells, T cells **cannot** undergo hypermutation to change the affinity of their TCRs for their cognate antigen.
- Traditional T cells also express either CD4 or CD8 “**co-receptor**” molecules. The ones that have CD8 recognize MHC I and are called cytotoxic T lymphocytes (CTL) or killer T cells. The ones that have CD4 recognize MHC II and are called T helper cells (Th)

- The αβ TCR has a specific extracellular domain that can bind to its ligand (the combination of MHC molecule and peptide), but the cytoplasmic tails of the α and β proteins are only about three amino acids long, this is too short and insufficient to transmit signal to the nucleus

- the αβ chain are great biding sites but they can not transmitting the signal across the cytoplasm. That's why the TCR is attached to a complex of proteins collectively called CD3.
- **This signalling complex (CD3)** is made up of four different proteins: γ, δ, ε, and ζ (gamma, delta, epsilon, and zeta).
- The CD3 proteins are anchored in the cell membrane, and have cytoplasmic tails that are long enough to signal.
- Please note, that the γ and δ proteins that are part of the CD3 complex are not the same as the γ and δ proteins that make up the γδ T cell receptor.

TCR



TCR signaling

- As with BCRs, signaling involves **clustering** TCRs together in one area of the T cell surface.
- When this happens, a threshold number of **kinase enzymes** are recruited by the cytoplasmic tails of the CD3 proteins, and the activation signal is dispatched to the nucleus.
- The tyrosine kinases activate cytoplasmic motifs (areas) which are termed ITAMS (this theme is similar to that in B cells and NK)

Co receptors of T cells

- In addition to the T cell receptor, there are two more molecules which are involved in antigen recognition by T cells – the CD4 and CD8 co-receptors.
- These co-receptor molecules serve two functions:
- 1. They **strengthen the adhesion between the T cell and the antigen** presenting cell to help focus the attention of Th cells and CTLs on the proper MHC molecule.
- 2. CD4 and CD8 also are **signaling molecules** just like the CD3 complex of proteins. Both CD4 and CD8 have tails that extend through the cell's plasma membrane and into the cytoplasm, and both of these tails have the right characteristics to signal. In contrast to CD3 molecules, which are glued rather tightly to the αβ T cell receptor on the cell surface, the CD4 and CD8 co-receptors usually are only loosely associated with the TCR/CD3 proteins.

Co

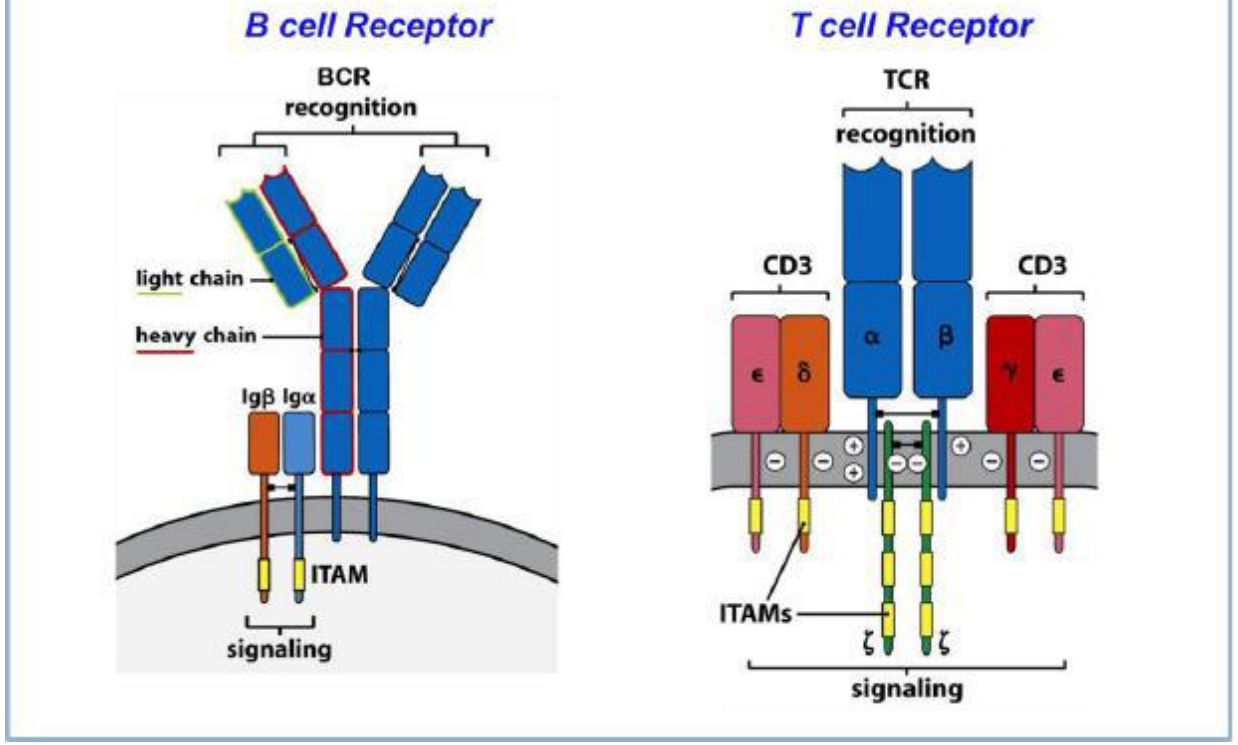
- Like B cells, T cells need costimulation to be able to send a strong enough signal to the nucleus.
- The first thing needed is aggregation (**cross-linking)** of TCRs.
- The second signal can come from different molecules but the best studied examples are the **B7** proteins (B7-1 and B7-2) which are expressed on the surface of antigen presenting cells.
- B7 molecules provide co-stimulation to T cells by plugging into receptor molecules called CD28 on the T cell's surface.
- Note that experienced T cells have a reduced requirement for co-stimulation.

- During an infection, a single activated T cell can give rise to about

10 000 daughter cells during the first week or so of proliferation (clonal expantion).

- This proliferation is driven by growth factors such as **IL-2**.
- Naive T cells can make some IL-2, but they don’t have IL-2 receptors on their surface – so they can’t respond to this cytokine.
- In contrast, activated Th cells produce large amounts **of IL-2,** and they also express receptors for this cytokine on their surface. As a result, newly activated helper T cells stimulate their own proliferation in an autocrine fashion.

T Cell and B Cell Antigen Receptors
(TCR and BCR)



Comparison between BCR and TCR

- . BCRs and TCRs both have “recognition” proteins that extend outside the cell, and which are diverse because they are made by mixing and matching gene segments.
- For the BCR, these recognition proteins are the light and heavy chains that make up the antibody molecule. For the TCR, the molecules that recognize antigen are the α and β proteins.
- TCRs and BCRs have cytoplasmic tails that are too short to signal recognition, so additional molecules are required for this purpose. For the BCR, these signaling proteins are called Igα and Igβ. For the TCR, signaling involves a complex of proteins called CD3.

Comparison/ continued

- For B and T cells to be activated, their receptors must be clustered by antigen, because this crosslinking brings together many of their signaling molecules in a small region of the cell. When the density of signaling molecules is great enough, an enzymatic chain reaction is set off that conveys the “receptor engaged” signal to the cell’s nucleus. These enzymatic reactions involve phosphorylation of the ITAM motifs within the cytoplasmic tails of the BCR and TCR. This phosphorylation occurs via tyrosine kinases.

Comparison/3

- Although crosslinking of receptors is essential for the activation of B and T cells, it is not enough. **Naive B and T cells also require co-stimulatory signals that are not antigen specific**.
- This two-signal requirement for activation sets up a fail-safe system which protects against the inappropriate activation of B and T cells.
- For B cell activation, a helper T cell can provide co-stimulation through surface proteins called CD40L that plug into CD40 proteins on the B cell surface. B cells also can be co-stimulated by “danger signals,” including invader specific molecular signatures or battle cytokines.
- For T cells, co-stimulation usually involves B7 proteins on an activated dendritic cell that engage CD28 proteins on the surface of the T cell.

Th1: T helper 1

- T helper cells type 1 produce: TNF, IFN-γ, and IL-2.
- The T helper becomes a type 1 helper when dendritic cells (DC) produce IL12, this happens when DC are stimulated by recognizing bacteria or virus .When the IL-12-producing DC presents the antigens to T helper, that Th cell will be instructed to become a helper type 1 which will produce:
- 1.TNF which activates macrophages and natural killer cells.
- 2. IFN-γ : strong macrophage activator and it also influences B cells during class switching to produce IgG antibodies which are especially good at opsonizing viruses and bacteria and at fixing complement.
- 3. IL-2 : stimulates NK cells and is a growth factor which stimulates the proliferation of CTLs, NK cells, and Th1 cells themselves

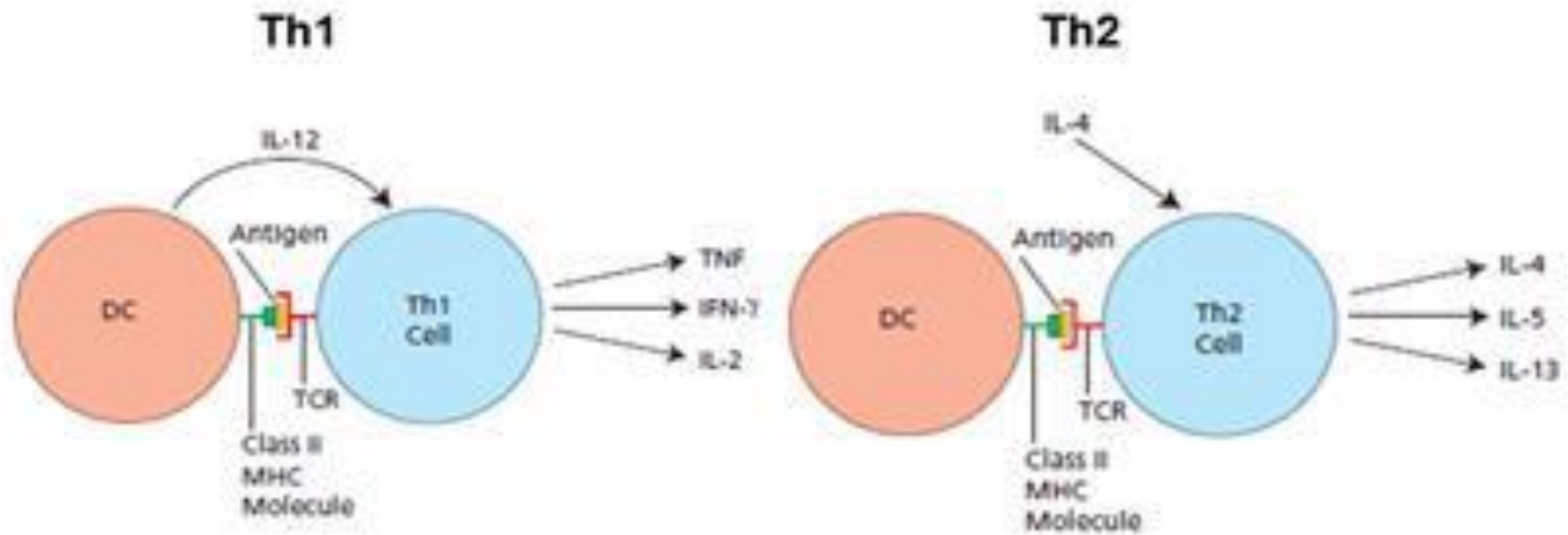
Th2 HELPER T CELLS

- These produce: IL-4, IL-5, and IL-13
- In case of parasitic infection or allergic reaction dendritic cells that recognize these two situations will activate those helper T cells which have T cell receptors that can recognize the antigen presented by the DC. This interaction results in helper T cells which are “programmed” to produce the Th2 subset of cytokines, which includes IL-4, IL-5, and IL-13.

Th2 continued

- 1. IL-4 : growth factor that stimulates the proliferation of Th2 cells (autocrine effect)
- IL-4 also is a growth factor for B cells, and this cytokine can influence B cells to class switch to produce IgE antibodies , which is the AB needed in these situations (parasitic infection and allergy)
- 2. IL-5 is a cytokine which encourages B cells to produce IgA antibodies, antibodies that are especially useful against bacteria which invade via the digestive tract. IL 5 also stimulates eosinophils.(remember that eosinophils are the main inflammatory cells involved in allergic reactions and parasitic infestation)
- 3. IL-13 stimulates the production of mucus in the intestines. This helps prevent more intestinal parasites or pathogenic bacteria from breaching the intestinal barrier and entering the tissues

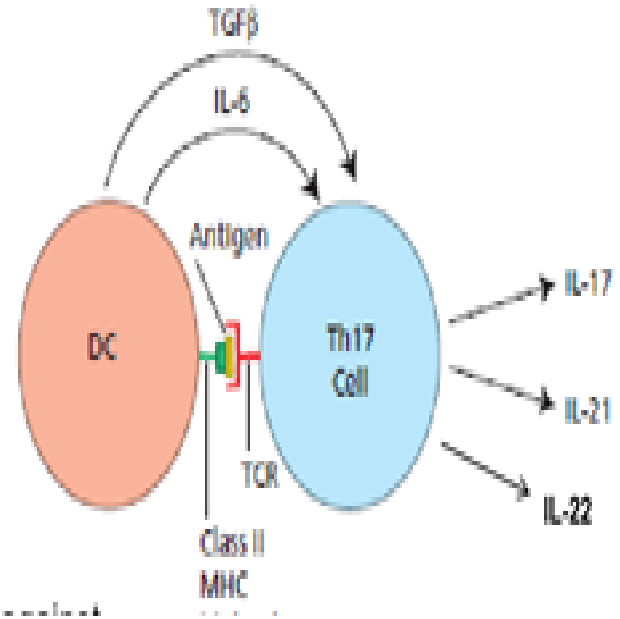
Th Subsets


Th1
IL-12
Antigen
DC
Th1
Cell
TCR
Class II
MHC
Molecule
TNF
IFN-γ
IL-2
Th2
IL-4
Antigen
DC
Th2
Cell
TCR
Class II
MHC
Molecule
IL-4
IL-5
IL-13

Th 17

- Th17 cells are needed in the defense against fungi .
- If a dendritic cell is present in an area of the body which is being attacked by fungi (e.g., a vaginal yeast infection) or by certain extracellular bacteria, that DC will produce TGFβ and IL-6, which together with co-stimulatory molecules, influence newly activated helper T cells to produce the Th17 subset of cytokines, which includes IL-17 and IL-21.
- 1. IL-21 encourages uncommitted Th cells to become Th17 cells, and this increases the number of Th17 cells available to battle the fungus.
- 2. Secretion of the “signature cytokine,” IL-17, results in the recruitment of massive numbers of neutrophils to the site of infection. These neutrophils help defend against pathogens against which Th1 and Th2 cells are relatively ineffective, including fungi and some extracellular bacteria .
- Patients who have a genetic defect in IL-17 secretion suffer from devastating fungal infections

Th17 cells



- Th17 also produces IL-22 which stimulates production of antimicrobial peptides and serves to maintain epithelial barrier integrity

Non traditional T cells

- Any T cell that doesn’t express αβ receptor is called a non-traditional T cell. There are mainly 2 types of these cells:
- 1.T cells which have γδ receptors, these are characterized by the following:
- A. γδ T cells **do not express the CD4 or CD8 co-receptor** molecules.
- B. they are **most abundant in areas like the intestine, the uterus**, and the tongue, which are in contact with the outside world.
- C. γδ receptors are **much less diverse** than αβ receptors.
- D. the receptors of γδ T cells in the tongue and uterus tend to favour certain gene segments during rearrangement, whereas γδ receptors in the intestine prefer other combinations of other gene segments. These γδ T cells stand watch on the “front lines,” and have receptors which are “tuned” to recognize invaders that usually enter at certain locations.

γδ T / continued

- E. although γδ T cells also are found in the thymus, it is not known exactly where they mature.
- F. it is not exactly known what the receptors on γδ T cells recognize, but it is thought that they recognize un-presented antigen. The receptors of some γδ T cells recognize proteins (e.g., MICA and MICB) which are expressed on the surface of cells that are under stress. Consequently, it has been postulated that γδ T cells are designed to kill cells that become stressed as the result of a microbial infection. However, the exact function of γδ T cells is not clear.

NKT.. Another example of non-traditional T cells

- NKT cell. In a human, only about 1% of the T cells in the blood are of this type. As its name implies, this non-traditional T cell has some of the properties of the natural killer (NK) cells of the innate system, and some of the properties of traditional T cells of the adaptive immune system.
- NKT cells mature in the thymus and have αβ receptors. However, in contrast to the αβ receptors of traditional T cells, which are incredibly diverse, the receptors expressed by NKT cells is quite limited. In addition, the receptors of NKT cells recognize lipids presented by non-classical, CD1 MHC molecules instead of protein fragments presented by class I or class II MHC molecules.
- These cells probably help in protection against microbes like tuberculosis which produce characteristic lipid molecules.

Secondary lymphoid organs..

- All secondary lymphoid organs share one histological feature: They all contain lymphoid follicles, which are critical for the functioning of the adaptive immune system
- Lymphoid follicles start as “primary” lymphoid follicles: loose networks of follicular dendritic cells (FDCs) embedded in regions of the secondary lymphoid organs that are rich in B cells

- The function of follicular dendritic cells is to **display** antigen to B cells. (note: they display the antigen which means they make it easier to be seen by the B cells, they don’t present the antigen because B cells do not need Ag presentation. This means B cells can directly recognize the antigen but FDC put the antigen in an accessible location)

High endothelial venules

- A second feature common to all secondary lymphoid organs except the spleen is the high endothelial venule (HEV). Most endothelial cells flat cells that are tightly “glued” to the cells adjacent to them to prevent the loss of blood cells into the tissues. In contrast, within most secondary lymphoid organs, the small blood vessels that collect blood from the postcapillary venules are lined with special endothelial cells that are columnar. These tall columnar cells are the high endothelial cells.

MALT

- Mucosa associated lymphoid tissue is composed of lympoid aggregates (lymph nodules) under mucosal surfaces. Peyer’s patches are examples of MALT which function as secondary lymphoid organs. Peyer’s patches begin to develop before birth, and an adult human has about 200 of them.

- Peyer’s patches have high endothelial venules through which lymphocytes can enter from the blood, and outgoing lymphatics that drain lymph away from these tissues.
- However, there are no incoming lymphatics . So the Ag can not enter throgh lymphatics because they are not available. Antigens enter through cells within the epithelium called M cells..
- These M cells are not coated with mucus, so they are easily accessible to microorganisms that inhabit the intestine. They are “sampling” cells which specialize in transporting antigen from the interior (lumen) of the small intestine into the tissues beneath the M cell. To accomplish this, M cells enclose intestinal antigens in vesicles (endosomes). These endosomes are then transported through the M cell, and their contents into the tissues that surround the small intestine. Except for its unusual method of acquiring antigen, a Peyer’s patch is quite similar to a lymph node.

- GOOD LUCK