Sheet 11 and 12: IMMUNO

Dendritic Cells:

- Most potent APC's.
- Express CCR7 chemokine receptors which help the dendritic cell migrate to the T cell rich zones. Ligands for these receptors (Homing receptors) are secreted (CCL19, CCL21)

MHC 1: for intracellular antigens

1. Proteins are tagged with ubiquitin
2. This leads to protein degradation by the proteasome into peptides
3. Meanwhile MHC1 is being synthesized in the ER
4. These peptides are taken into the ER using TAP transporter
5. Tapasin binds to TAP in order to release the peptide from TAP for it to bind to MHC 1 in the ER
6. The MHC 1 complex is now taken to the Golgi apparatus
7. It is then sent through exocytotic vesicles tot he cell surface

MHC 2: For extracellular antigens

1. Proteins are taken into the cell by endocytosis into endosomes
2. The endosome becomes a phagosome
3. It then fuses with a lysosome to from a phagolysosome
4. The lysosomal enzymes (cathepsins) degrade the proteins into peptides
5. Meanwhile the MHC 2 is being synthesized in the ER
6. The MHC 2 immediately has an invariant chain bound to its cleft (preventing MHC1-associated peptides from binding to MHC2)
7. It is then taken to the endosome where the chain is trimmed to form CLIP
8. HLA Human leukocyte antigen is used for the exchange between the CLIP and the peptides

B cells	T cells
Recognize a larger variety of antigens	Have MHC restriction - only recognize linear peptides presented with MHC molecules -
B cell Receptors may be membrane bound or in their soluble form (antibodies)	Receptors are always membrane bound (TCR)
Have effector functions done by the Fc portion of the antibody (complement activation, opsonization and phagocyte binding)	No effector functions
Signal transduction: Iga (alpha) and Igb (beta)	Signal transduction: CD3 and Zeta

ANTIBODIES :

Heavy chain:

1 var domain and 3 constant domains (IF SOLUBLE)
1 var domain and 4 constant domains (IF MEMBRANE BOUND)

Light Chain:

1 var domain and 1 constant domain

TCR:

1. Alpha chain:

- 1 Var domain and 1 constant domain

2. Beta chain:

- 1 Var domain and 1 constant domain

3. Transmembrane sequence

———(or gamma delta)———

TCR Complex: Complex of TCR and associated signaling molecules (CD3 AND ZETA)

BCR complex: Complex of BCR along with Igalpha and Igbeta (signaling molecules)

- Lymphocyte development:

1. Common lymphoid progenitor cells commit to either B cell lineages or T cell lineages.
2. Proliferation and antigen receptor gene rearrangement (rearrangement of the segments that code for variable regions of the receptors)
3. Positive or negative selection

Diverse antigen receptor

1. Genes coding for the v region
2. Genes coding for c region
3. Between the two regions, there are Diversity D and Joining J gene segments. All receptor gene loci have V J C genes. Ig heavy chain and the TCR beta chain contain D gene segments too.

B cell Maturation:

1. Bone marrow progenitors that are committed to B cell lineage give rise to B cell precursors (Pro B cells) - these dont express BCRs)
2. The pro B cells start to express BCR's and become pre B cells which express BCR's (express IgM)
3. Pre B cells are immature B cells
4. mRNA that expresses or is transcribed to give m heavy chains, is spliced giving mRNA that is transcribed to give IgM and IgD
5. The cell is now ready to respond to antigens
6. Negative and positive selection:

- Positive selection: Functional BCR expressing B cells are selected
- Negative selection: B cells that strongly react to self antigens are unselected

T cell Maturation:

1. T cell progenitors migrate from the bone marrow to the thymus to mature
2. T cell progenitors then form **pro T cells** which do not express TCR's- **also called double negative T cells-**
3. The first thing that happens is the beta chain expression of the T cell receptors- it is now called a **Pre T cell** (expresses beta chain).

- This occurs by recombination mediated by VDJ recombinase.
- If recombination is successful in one of the two inherited loci is successful then the beta chain will be expressed on the surface of the cell and the pre TCR complex is formed which sends signals to promote 1. Survival 2. Proliferation 3. Alpha chain gene recombination
- If the recombination is unsuccessful on one of the loci then the recombination takes place on the second. If recombination is unsuccessful on both of them, then the TCR beta chain is not produced in a pro T Cell, it is not able to form a PRE T cell and the T cell dies
- The signal released once the pre TCR complex is assembled inhibits the VDJ recombination at the second TCR beta chain locus (Allelic exclusion), and leads to alpha chain gene recombination. Failure to express the alpha chain and the COMPLETE TCR results in the death of the cell.

4. Alpha chain is expressed so that the complete abTCR is expressed (THE CORECEPTORS CD8 AND CD4 are EXPRESSED HERE) - it is now called a **Double positive T cell** -

5. Positive or negative selection of T cells occurs: (according to how they react to self MHC molecules)

- IF TCR of a T cell recognizes an MHC molecule and interacts with low to moderate affinity then is it SELECTED TO SURVIVE (Positive Selection)
- IF the TCR strongly interact with the MHC molecule, then the cell undergoes APOPTOSIS (negative selection)

6. Selection of the mature T cells from double positive to single positive:

- T cells whose TCR's recognize MHC1 peptide complexes —> Become CD 8 cells
- T cells whose TCR's recognize MHC2 peptide complexes —> Become CD 4 Cells

Sheet 13:

Antigen entry:
1. In tissues:
Antigens (free antigens or carried by APCs) —> Lymph nodes

2. In the blood:
Antigens —> Spleen

In the LN (lymph node):

B cells reside in:
Follicles by following CXCL-13

T cells reside in:
Paracortex by following other chemokines

In the Spleen:

B cells reside in:
Follicles and the marginal zone of white pulp

T cells reside in the:
PALS (Para Arteriolar Lymphoid sheath)

Mature B cells:
- Have completed maturation in the secondary lymphoid organs and migrate from one secondary lymphoid organ to the next
- Have both IgM and IgD
- Mature NAIVE B cells: Have both IgM and IgD but haven't come across a cognate antigen yet.They remain in the follicles waiting for an antigen(NO ANTIGEN > DEATH)

Immature B cells:
- Havent completed maturation in the secondary lymphoid organs

Mature T cells:
- Are mature as soon as they leave the Thymus
- Are Naive until they are activated by dendritic cells

B cell response:

- 1 : B cells entry into the follicles
1. Follicular Dendritic Cells (FDC) release CXCL13 chemokine to attract the B cells to the follicles
2. CXCL13 binds to CXCR5 receptor on circulating B cells, attracting them to the follicles.
3. Once the B cells are in the follicles, they wait for the antigens to reach them in time

B cell survival depends on:

1. Whether the B cell comes in contact with its cognate antigen or not: the antigen binds to the immunoglobulins on the b cell surface, prolonging the B cells survival via signals.
2. Inputs received from BAFF cytokine (B cell activating factor of TNF family) / BLyS (B lymphocyte stimulator), provide maturation and survival signals.

- 2 : Antigen delivery to B cells

In all of the following cases, antigens are delivered to B cells as they are and are not converted or altered (unlike t cells) :

1. Soluble antigens: pass through conduits (passages) to reach the B cell follicle from the subcapsular sinus
2. Medium sized antigens: Are taken to the follicles by the resident dendritic cells in the medullary region
3. Large Antigens (Opsonized, large, or antibody antigen complexes) : Are captured by the subcapsular macrophages in the subcapsular sinus and are taken to the follicle. CD3 binds to CD2 Complement receptors

- 3: Activation of B cells

For FULL strength of induction and response or activation, what's needed is:
1. BCR complex: Ig (for antigen binding), and IgAlpha & IgBeta (to relay the signal)
2. Complement proteins
3. Pattern Recognition Receptors (ex: TLR's)

For weaker signaling and partial activation, not all the molecules mentioned above are needed…

If antigen is a protein:
It is internalized into an endosomal vesicle to be processed and presented onto B cell surface for T helper cell recognition

If antigen is not a protein:
Its binding onto the surface is sufficient enough for the signal.

- 4: B cell response to antigens:

1. T cell dependent: if the antigen is a protein and it is presneted onto the B cell surface for T cell recognition
2. T cell independent: Non protein antigens which do not need T cells to recognize them for the response to occur. They display multiple identical epitopes to cross link many B cell antigen receptors, and initiate a response without the need for T cells

B cell subsets:
1. Follicular B cell: usually mature B cells called recirculating b cells due to their migration from one lymphoid tissue to another, that release IgM and IgD.

2. B1 B cells:
- Release IgA at mucosal sites
- Release IgM spontaneously against polysaccharide and lipid antigens
- Derived from HSC of the fetal liver

3. Marginal zone B cells:
- Respond to polysaccharide antigens to generate natural antibodies

Effects of antigen binding on the B cell:
Resting cells enter the G1 stage of the cell cycle:

1. Increase in cell size
2. More cytoplasmic RNA
3. Antiapoptotic proteins released (BCl2) for survival
4. Cell proliferation
5. Increase expression of B7-1 and B7-2 (ligands for T cell receptors)
6. Increased expression of CCL17

T cell dependent antibody response to protein antigens:

T cells and B cells must find each other and physically interact for a strong antibody response.

1. T cells:

Helper T cells are activated by antigens, and are induced to proliferate:

- They express CD40L which is the ligand for CD40 on b cells
- They express more CXCR5 which is the receptor for FDC CXCL13. This helps CD4+ T cells migrate towards the follicle
- Down-regulate Chemokine receptor CCR7
- Secrete cytokines

2. B cells:
- Down-regulate CXCR5
- Expresses CD40 Receptors for the CD40L on T cells
- Expresses CCR7 which is attracted towards the ligands CCL19 and CCL21

Hapten-carrier effect:
- Sugars or lipids that are not immunogenic by themselves and need a protein to bind to it and act as a carrier (remember that protein antigens always give rise to the strongest response- the T dependent response-)
- This Hapten-carrier immune response is helpful in conjugate vaccines. (which consists of a polysaccharide antigen (hapten) conjugated to a carrier molecule)

Sheet 14

Bone marrow has hematopoietic stem cells:
HSC -> Progenitor Lymphoid Cells -> Immature B cells (by somatic recombination)

Progenitor B cell —(somatic recombination of heavy chain)—> Precursor B cell
Precursor B cell —(somatic recombination of light chain)—> Immature B cell

HEAVY CHAIN GENE:

Consists of:
1. Leader segment
2. C Constant region (Cμ for IgM)
3. V Variable region
4. D Diversity segment
5. J Joining segment

- V and D are far away from each other
- J and C are in close proximity

Mitotic division of hematopoietic stem cells (and gene recombination)

1. DJ Recombination:
In order to get the D and J segments closer to each other and for them to bind, pieces between them are cut (Parts of the segments could be cut as well) . This brings the C in close proximity. (Cμ has many segments, for IgM we have 4 constant regions since the heavy chain of membrane bound Ig has 4 constant regions

(IF FREE AND NOT MEMBRANE BOUND IT WOULD HAVE BEEN 3)
<< ref. To page 2 if you're confused by this

2. **VDJ recombination:**
Gets V segment closer to D and J, allowing them all to bind.

3. We end up with a **daughter B lymphocyte (with 1V 1D 1J segments)** when originally we had (1-40 V 1-23 D and 1-6 J)

This gives rise to millions of different combinations and possibilities, which means itll give rise to millions of different variable regions.

4. This sequence is then **transcribed** to pre mRNA
5. The pre mRNA is **spliced**- introns are removed-, forming the mRNA
6. The mRNA is **translated** to form the HEAVY CHAIN OF THE IG (IgM)

Next, we will move on to light chain gene segment recombination..

Two types of light chains:
1. Lambda
2. Kappa

Both of them dont have the D Diversity segment

Kappa light chain rearrangement:

Kappa light chain gene consists of:
1. 1-38 V regions
2. 1-5 J segments
3. 1 Ck (Kappa constant)

Here, we have V J and C, not VDJ and C (NO D)
- J and C are in close proximity
- V is far away form them

What happens is:

1. VJ recombination, where V is Brought closer and binds to J (new nucleotides can be added in this step)
2. This brings the C region closer to VJ
3. This is transcribed to RNA
4. Splicing of RNA
5. We end up with 1V 1J and 1C

———————————-

- We have different constant regions when class switching occurs

- During recombination, new nucleotides may be added to give rise to increased specificity and diversity of the Ab. —> Hyper-variable regions (CDR- complementarity determining regions)

- Different Ig's have different variable regions

- We have hyper-variable regions on both heavy and light chains (total of 3 CDR's)

RSS (recombination signalling sequence) -

- Sequences present between V(D)J segments
- RSS is used in recombination initiation by 1. deletion or 2. inversion
- RSS is composed of:
1. Heptamer (7 nucleotides)
2. Spacer (12 or 23 bp)
3. Nonamer (9 nuclotides)
- It exhibits the 23/12 rule where a 23 and 12 CAN bind but 12 and 12 cannot, and 23 cannot recombind with 23 either.
- So if two V segments have 12 bp each, they cannot bind to each other since they both have 12 bps.
- However, if one had 12 and a J segment had 23, then they can bind to each other.

1. Deletion (hairpin loop configuration)

- Let's say we want to join V segment to a J segment (where they follow the 23/12 rule)

1. The 12 and 23 bp spacers will lie parallel to each other forming a HAIRPIN LOOP
2. RAG1 and RAG2 identify the RSS
3. They break the DNA double strand in both V and J and break the RSS
4. Ku80 and Ku70 proteins bind to the V and J segments
5. Ku proteins initiate repair by forming the hairpin loop where RAG broke the RSS
6. DNA protein kinase (artemis) opens the hair loop
7. TDT protein adds in the nucleotides into the separated V and J segments randomly
8. DNA ligase ligates the ends together
9. Forming a unique and repaired V and J recombinant segment

2. Inversion (Tangled configuration)

1. During VJ recombination, V and J are brought closer to each other in a way that tangles the DNA
2. RAG proteins will cut at the RSS
3. No deletion occurs, but it is rather inverted

Sheet 15

T-dependent B cell response:

1. Dendritic cell catches the antigen, processes it and presents it on its surface for the T helper cell
2. The dendritic cell goes to the LN to present the antigen to the T helper cell, activating it.
3. Once activated, the T helper cell changes its expression of chemokine receptors (INCREASE CXCR5 and DOWN-REGULATE CCR7) to migrate to the follicle where the B cells are, and follows the chemokines coming from the follicle.
4. Within the follicle, there are antigens in their native form (protein antigens) that will bind to Ig's on the surface of the B cell
5. This partially activates the B cell. The B cell reaches a certain level of activation but not its full potential.
6. The antigens are processed and presented on the B cell surface with an MHC II molecule.
7. The B cell then changes expression of chemokine receptors (INCREASE CCR7)
8. The B cell leaves the follicles to meet with the T helper cell
9. The antigen-MHC II complex is presented to the T helper cell
10. This activates the T helper cell, making it express CD40 ligand, which binds to CD40 receptors on B cells.
11. The T cell also releases cytokines which bind to cytokine receptors on the B cell surface.
12. This activates the B cell FULLY
13. Once the cytokines and CD40 bind to the B Cell cytokine receptors, this initiates something called the Extra-follicular focus
14. This leads to proliferation of B cells to produce **short-lived plasma cells** that produce mostly IgM.

15. The B cell then returns to the follicle, along with the T helper cell.
16. The T helper cell in the follicle is now called Follicular T Helper cell.
17. **Germinal center** forms and contains:
    1. Follicular T helper cell
    2. Activated B cell clones (coming from the same B cell that interacted with the T cell)
    3. Follicular dendritic cells.
18. What happens in the germinal center is: Proliferation and differentiation of the B cells to form **LONG LIVED plasma cells and MEMORY CELLS.**

(If you're confused, go over it again. Trust me, it's really simple you just need to focus and try to imagine what's happening as you read)

So remember,
short lived plasma cells —> produced outside of the follicle
Long lived plasma cells —> Produced in the germinal center in the follicle

What happens in the germinal center:
1. Affinity maturation (generate antibodies with better affinities)
2. Isotype switching (IgM-> A,G,E)
3. Memory B cell generation
4. Long lived plasma cell differentiation

REMEMBER: before the formation of the germinal center, we had the PRIMARY follicle. Primary follicle had: 1. Naive B cells , and 2. Follicular dendritic cells

Germinal center is divided into:

1. Light zone: lesser cells
2. Dark zone: densely packed with proliferating cells

Immunofluorescence: (label cells)
1. Green stain (used anti CD23 antibodies)
   - detect CD23 containing naive B cells outside the germinal center

Dim green color outside the germinal center

2. Bright green (light zone)
   - Detects follicular dendritic cells (higher density of CD23)

3. Red (dark zone) :
   Detects mitotically active B cells. Some of them migrate to the light zone giving a yellow stain. (mix between green and red)

green-> cells that express CD23
red-> highly mitotic

FDC's:
- Have receptors (CD1 CD2 CD3 and Fc receptors) to bind to antigens that deposit fragments of complement proteins (C3d and C3b, etc)
- Fc receptors are needed for the FDC's to bind to the antigens bound to antibodies
- Only found within the follicle

- Dont need MHC molecules to present the antigens. They present the antigens in their native conformation to B cells.

Why do we need the FDC's to present the antigens to the B cells if the b cells are already activated?? For affinity maturation and isotype switching.

How?

1. Some activated B cells leave the dark zone and migrate to the light zone where they:
    1. Interact with antigen in their native conformation presented by the FDC's through the Fc and complement receptors
    2. By expressing MHC II-antigen complexes on the B cell surface, and presenting them to the Follicular T Helper cells. —> cytokines are released from the follicular T helper cell

2. Either way, this leads to the release and production of higher affinity antibody-secreting memory and plasma cells. (affinity maturation and isotype switching)

3. The plasma cells and memory cells exit the germinal center

Isotype Switching: (occurs in the light zone)

Whether the the immunoglobulin switches from IgM to IgE or A depends on:
1. The cytokines released by the T Helper cell, and this depends on the type of microbe that initially activated the B cell.
2. Location

According to microbes:

1. Bacteria and viruses: (IFNγ SWITCHES IGM TO IGG)

In this case TH1 cells secrete **IFNγ** which leads to switching from **IgM to IgG**, to fihgt against this long lasting infection.

REMEMBER:
- Polysaccharide antigens can activate the B cells alone without the help of the t cells (T INDEPENDENT).
- B cells are activated by the antigens alone by cross-linking receptors together and producing a signal strong enough to stimulate the B cell to secrete IgM without the need for class switching by TH- cell.
- BUT when prolonged immunity is needed then class switching must occur to convert or switch between IgM and IgG through cytokine release by TH cells.

2. Helminthic infections and hypersensitivity/ allergic reactions

In this case **IgM switches to IgE b**y the release of **IL4** by TH2 Cells.

According to location:
MUCOSAL TISSUE
TH cells secrete **TGFβ, APRIL and BAFF** cytokines, switching from **IgM to IgA**

Affinity Maturation:

Occurs in two steps:

1. Somatic maturation of Ig Genes which occurs during activated B cell proliferation in the dark zone of the germinal center. Mutations in the V region occur. This produces HIGH AFFINITY antibodies and LOW AFFINITY antibodies
2. Selection of high affinity antibodies. The B cells with antibodies of different affinities migrate to the light zone where follicular TH cells release **IL21 cytokine which induces apoptosis of B cells**
   - **For high affinity B cells:** they will be saved by binding to an antigen presented by the FDC's in the light zone, and the B cell can also bind to follicular TH cells that release cytokines to keep these B cells alive. (SURVIVAL SIGNALS)
   - **For low affinity B cells:** Low affinity B cells wont be able to bind to the FDC's or the Follicular TH cells, they will be affected by the IL 21 cytokine, leading to apoptosis.

VACCINES:

- **Using live attenuated pathogens: STILLR REPLICATE BUT ARE WEAKENED**

How are they attenuated?

1. Passing the virus along a series of cultures (IN VITRO) (EX CHICKEN EMBRYO CULTURE). As you transfer the pathogens that successfully replicate and infect from one culture to another, the pathogen adapts best with cultures, but loses its ability to infect and replicate in the OG human host, but can still replicate and so on (IT BECOMES WEAKER)
2. Culture the pathogen at different temperatures. By allowing pathogens to replicate at temperatures Lower than that of the human body (EX 25 degrees). The pathogens that survive in that temp are moved to the human body (37 degrees) and arent as infective and pathogenic due to the temp change (WEAKENED)

Live attenuated vaccines are produced by altering the virus wild type (WILD TYPE —> ALTERED AND WEAKENED). This altered version of the virus cannot cause the disease that the wild type causes.

Live attenuated vaccines are usually produced from viruses rather than bacteria (viruses have fewer genes and attenuation is more controlled) and shouldnt be given to immunocompromised patients (RISK OF REACTIVATION)

BCG IS THE ONLY LIVE ATTENUATED BACTERIAL VACCINE, and works agains TB.

- OPV (Oral polio vaccine) is a live attenuated vaccine
- It is administered through oral drops
- Induces intestine mucosal immunity.
- Can rarely cause vaccine associated poliomyelitis due to mutation of the virus into a virulent form.

Inactivated vaccine

- non live vaccines (viruses or pathogens are killed by chemicals, heat and radiation)
- Are still immunogenic
- Safer than live attenuated viruses (for immunocompromised patients)

- Part of a nonliving (fragments)vaccine can be taken and this is called a subunit vaccine. Such fragments can be proteins, polysaccharides, or other parts of the virus that can form virus like particles (VLPs)
- How are subunit vaccines produced?

1. Obtain surface antigen and purify it using molecular techniques - Pertussis vaccine -
2. Take DNA from the pathogen and put it in a living expression system that produces more of the needed protein (EX yeast or E.Coli) - hepatitis B vaccine -

1. TOXOID VACCINE: a type of subunit vaccines:

Inactivated toxin, which has the antigenic determinant only (so it is a nontoxic toxin)

- IMP in bacteria that depend on toxins like: C. Difficile, C. Tetani and Corynebacterium
- They protect against pathogenesis in the vaccinated indivisual
- Unlike live attenuated vaccines, they do NOT provide Herd Immunity.
- They do not protect unvaccinated individuals from infection or transmission from a vaccinated individual. (TOXOID AFFECTS THE TOXIN NOT THE PATHOGEN ITSELF)

2. Polysaccharide and conjugate vaccines: non lived vaccine

- Protects agains encapsulated bacteria like
    1. Streptococcus pneumonia
    2. Hemophilus Influenza type b (Hib)
    3. N meningitidis
- Polysaccharides are taken form the capsule and are made into a vaccine, which is poorly immunogenic.
- Why? Because polysaccharide antigens induce a T cell independent response for B cells and this will lead to the production of IgM antibodies from short lived plasma cells
- What do we do to make them more immunogenic to provide more long lasting immunity? The vaccine is conjugated with proteins **(HAPTEN CARRIER EFFECT).**
- This gives us a T cell dependent response that induces high affinity Antibodies and long term protection

Vaccine drawbacks:

1. HIV and Hepatitis C viruses have antigenic hyper-variability (constantly changing)
2. Some pathogens have multiple serotypes
3. Pathogens that are found intracellularly cannot be detected by antibodies

Sheet 16

Remember:

Extra-follicular focus —> Short lived plasma cells
T cell independent response (against non protein antigens)—> short lived plasma cells
Germinal center —> Long lived plasma cells and long lived memory cells. (by affinity maturation)

Plasma cells:

- Terminally differentiated: no going back once they're produced.
- Committed to antibody production
- Generated once B cells are activated through signals from BCR,CD40,TLRs

2 types of plasma cells:
1. Long lived plasma cells:
2. Short lived plasma cells

	Long lived plasma cells	Short lived plasma cells
Location	Bone Marrow	- Secondary lymphoid organs - Peripheral non-lymphoid organs (skin)
How are they Generated	- T cell dependent response in germinal center of the follicle	- T cell independent response (mainly) - T cell dependent response in extra-follicular foci (SMALL amount only)
Antigen that induces its production	Protein antigen	Nonprotein antigen (lipid, etc)
	Are maintained by the BAFF family which keep giving them survival signal once they've gone past the IL 21 apoptosis (they survive)	

Changes during B cell differentiation:

1. Increase in cell size
2. Prominent ER for Ab production
3. Cell is transformed into a secretory cell
4. Change in Ig production: from membrane bound to secreted form
5. Cytoplasm to nucleus ratio increases

Memory cells:

- Survive for a long time, without the need to continue antigenic stimulation. This happens thanks to the production of antiapoptotic proteins (Bcl-2)
- Are not terminally differentiated, which means once they reencounter a specific antigen:

1. They reactivate very quickly
2. They propagate themselves
3. They create plasma cells
4. Re enter germinal centers for affinity maturation (FURTHER affinity maturation)

They either
1. Remain in the lymphoid organ
2. Or they exit germinal centers and recirculate

Antibody feedback:

Secreted antibodies inhibit continuing B cell activation, inhibiting further antibody production. How?

1. Secreted antibodies form anitgen-antibody complexes with the specific antigen
2. This then interacts with the B cell in a way that inhibits its activation
3. The antigen part of the complex binds to the antigen receptor on the B cell (BCR)
4. The antibody (secreted) part of the complex binds to the inhibitory **FcγR**
5. This brings inhibitory phosphatase close to the antigen receptors
6. This prevents phosphorylation, which prevents the transmission of the BCR signal responsible for activation
7. No activation—> No further production of antibodies

This helps when theres an abundant amount of antibodies and we don't want to produce any more of them.

Effector mechanisms of humoral immunity:

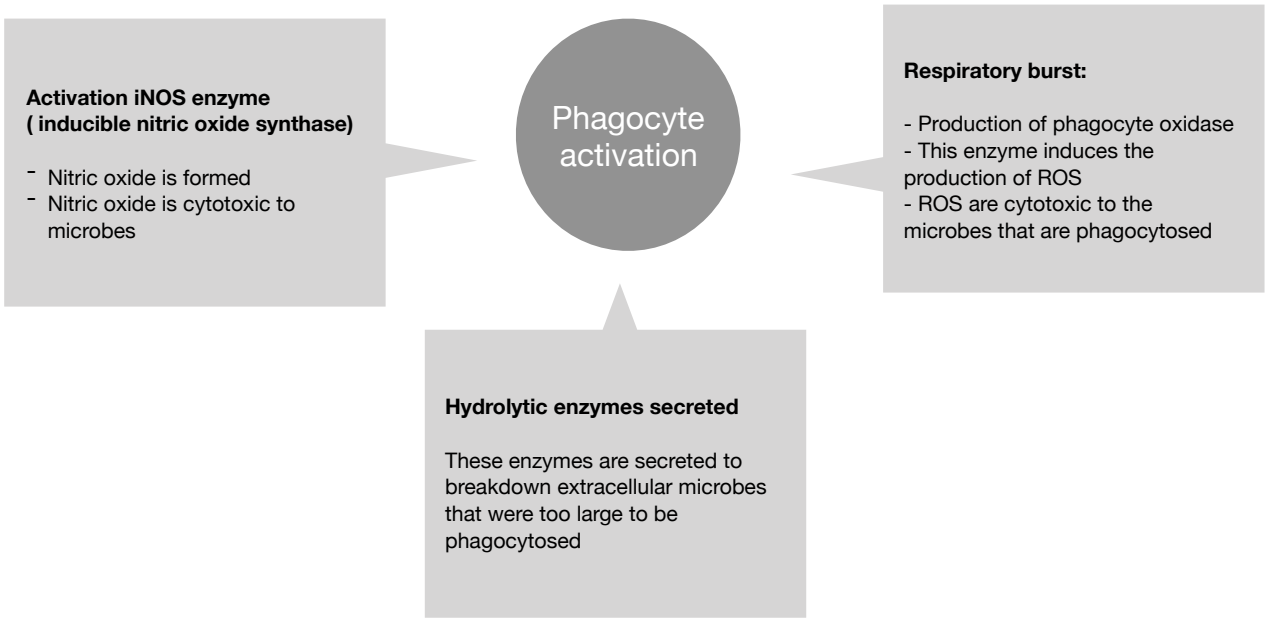
Consists of:
1. Antibodies
- neutralization of microbes and toxins:

By:
1. Binding to the antigen, preventing primary infection
2. Binding to the antigen, preventing spread of infection to adjacent cells
3. Blocking AB toxins from binding to their receptors on cells

- Phagocytosis and opsonization

Best opsonins: IgG3 and IgG1. These coat antigens as opsonins, and bind to **FcγR receptors** on phagocytes —> Engulfment of the microbe —> Activation of the phagocyte

The following figure shows what happens during phagocyte activation:



3. Antibody dependent cellular cytotoxicity:

- NK cells and leukocytes bind to antibody coated cells (infected cells) by binding to antibody Fc receptor, and destroy these cells.
- NK CELLS:

FcγR receptors engagement activates NK cells to secrete cytokines like IFNγ, and discharge NK cell contents of their granules (granzymes and perforins)
- This activation of NK cells also occurs when tumor cells EGFR's are targets for monoclonal antibodies. Once these growth factor receptors are targeted, their signals are blocked, and NK cells come in to save the day by antibody- dependent cellular cytotoxicity.

2. Complement system
3. Antimicrobial peptides

Helminthic clearance by antibody production:

This involves:

1. Eosinophils
2. Antibodies
3. Mast cells

What happens??

1. Antigens secreted by helminths are recognized by APC's
2. This acticates helper T cells
3. T cells secrete IL 4
4. IL4 promotes isotype switching of b cells to IgE releasing plasma cells
5. IgE will bind to the antigens on the helminth
6. It is then recognized by FcεR receptors on eosinophils
7. Degranulation of eosinophils
8. Cationic toxic proteins in eosinophil granules are released
9. They kill the helminth
10. meanwhile, mast cells are also degranulated by binding to the IgE
11. Mast cell mediators released induce Broncho-constriction and local motility (GI and gastric motility)
12. This helps with expulsion of the worms

Sheet 17

Immune tolerance (IT)

- The ability of the immune system to discriminate between self and non self antigens
- The body does not make antibodies against self antigens
- Lack of immunologic response to self antigens
- Failure of immune tolerance leads to autoimmune disease (AI)
- Tolerance is important to prevent unwanted immune response during transplantation
- Important for gene therapy, when immune response is prevented from occurring against newly expressed genes.

- **Two types:**

1. Central IT

- In central lymphoid organs (bone marrow and Thymus)
- Occurs when immature lymphocytes encounter self antigens.
- How the body deals with central IT:

1. Apoptosis of lymphocytes
   - Formation of pro apoptotic proteins and receptors
   - **Intrinsic pathway**: imbalance between BAX and BAK (proapop.) and Bcl2 and Bcl-XL (antiapop) —> Cytochrome C leakage from mitochondria —> Activate caspases
   - **Extrinsic pathway**: Fas ligand binds to Fas receptor on the apoptotic cell —> Activates caspases
2. Receptor editing: Change in receptors (B cells only): by increasing expression of RAG enzymes (Recombination activating genes) which change the light chain, preventing apoptosis of the B cells by negative selection.
3. Anergy (B cells only): long lasting functional unresponsiveness (Lymphocytes are viable but unresponsive and nonfunctional)
4. Development of regulatory T lymphocytes (CD4+)

Central T lymphocyte Tolerance CTLT

- Mechanisms for CTLT:

1. Apoptosis
2. T Reg cell formation

- Self antigens play a vital role in negative selection of T cells, and are triggered using (AIRE) Auto Immune REGulator proteins.
- Occurs in the thymus
- No anergy of T cells here

Central B lymphocyte Tolerance CBLT

-Mechanisms for CBLT:

1. Apoptosis
2. Anergy
3. Receptor editing: self reacting B cells escape apoptosis by expressing RAG which changes their light chain structure, aiding in the formation of a new receptor that doesn't react with self antigens

———————————

4. Peripheral IT

- In peripheral lymphoid organs
- Occurs when mature lymphocytes encounter self antigens.
- How the body handles it:

1. Apoptosis
2. Anergy (FOR BOTH T and B lymphocytes)
3. Suppression by regulatory T cells

Peripheral T lymphocyte Tolerance (PTLT):

- Mechanisms for CTLT:

1. Apoptosis
2. Suppression using T REG cells
3. **Anergy** (unlike CTLT which doesnt involve anergy for T cells)

Types of antigens:
- Immunogenic (induce immune response) and tolerogenic (induce immune tolerance where lymphocytes become inactive or are killed)

IPS (immune privileged sites) : Brain, testes and eyes
- Have no lymphatic drainage
- Are separated from antigens
- Involved in immunologic ignorance where lymphocyte IGNORES antigen presence.

Anergy:

- remember, in order to activate naive lymphocytes, two signals are needed:
1. Binding of an antigen to its receptor on the lymphocyte (free antigen for B cells or MHC bound for T Cells)
2. Co-stimulatory signal: achieved by binding of B7 proteins on APC's to their receptors on lymphocytes (CD28)

IF the co signal is weak:

- 1) T lymphocytes will engage the CD28 inhibitory receptors family (CTLA4 cytotoxic lymphocyte antigen 4 and CD152) or PD1 Programmed death proteins that induce apoptosis.
- Instead of having B7-CD28 activating signal, we will have B7-PD1 or B7-CTLA4 which induce apoptosis
- 2) the first signal activates ubiquitin ligase enzymes which target signaling proteins for degradation by proteases

T REG CELLS:

- T REG Cells regulate immune response.
- T REG Cells express CD25 and FoxP3 which is a transcription factor
(CD25 is a receptor for IL2 which is responsible for T cell activation and proliferation)
- T reg cells suppress immune responses by:
1. Production of some cytokines IL10 and TGFbeta which inhibit lymphocytes, DCs and macrophages
2. Increase expression of CD25, reducing the IL2 levels
3. Expression of CTLA4 and PD1 which bind to B7 molecule.

AI (AutoImmunity)

1. Organ specific: against one or few organs
2. Systemic: against multiple tissues

- Multifactorial: Depends on
    1. Failure of tolerance
    2. Environmental factors

- Infections: Break T cell tolerance

- How? Some microbes have antigens that are similar to self antigens or cross react with self antigens. This means that the immune response against these microbes will attack self antigens as well.

3. Genetic Factors (multiple gene loci especially MHC loci)

B Single-gene defects that cause autoimmunity (mendelian diseases)

Gene(s)	Disease association	Mechanism
AIRE	Autoimmune polyendocrine syndrome (APS-1)	Reduced expression of peripheral tissue antigens in the thymus, leading to defective elimination of self-reactive T cells
CTLA4	Autosomal dominant immune dysregulation syndrome	Impaired inhibitory checkpoint and regulatory T cell function leading to loss of B and T cell homeostasis
FOXP3	Immune dysregulation, X-linked polyendocrinopathy and enteropathy (IPEX)	Deficiency of regulatory T cells
FAS	Autoimmune lymphoproliferative syndrome (ALPS)	Defective apoptosis of self-reactive T and B cells in the periphery

Remember:

CD25: T cell receptor that binds to IL2 for T cell activation
CD28: Binds to B7 protein for T lymphocyte activation (co stimulatory signal)
FoxP3: Transcription factor expressed by T reg cells
PD1: Induces apoptosis (programmed death) by binding to B7 protein (CD28 inhibitor)
CTLA4: Cytotoxic lymphocyte antigen 4, CD28 inhibitor
BAK AND BAX: Pro apoptotic proteins
Bcl2 and Bcl-XL: Anti apoptotic protein
FasL: binds to Fas receptor, inducing apoptosis by activating caspases
CD3: Signal transduction by TCR complex
Zeta: Signal transduction by TCR complex
CD4: binds MHC class II
CD8: binds MHC class I
LFA-1: Adhesion molecule (binds to ICAM 1 on APC)
IL12: Induces conversion of naive T helper cells to TH1 cells
IL4: induces conversion of Naive T cells to TH2 Cells
IL5: important for recruitment of eosinophils
TH1: release IL-2 to activate CD8 cells, and release IFNγ to activate macrophages and for B cell isotype switching from IgM to IgG
TH2 (CD23) : Releases IL-4 and IL-5 for antibody mediated immunity (production of plasma cells from B cells)
IL2: produced when CD28 and B7 bind to each other. VITAL step in T cell activation
FC epsilon R2: On TH2 cells, bind to IgE and antigen to indulce late phase of hypersensitivity
FC epsilon R1: induces primary or early phase hypersensitivity (found on mast cells, basophils and some eosinophils) by binding to IgE
IFNγ: Released by Th1 cells to activate phagocytes and induce their microbicidal properties
Calcineurin: Protein enzyme that switches IL-2 genes ON

Sheet 18

Cell mediated Immune response:
Brought about by two types of infections:
1. Microbes that are phagocytosed but are resistant to microbicidal activity of phagocytes
2. Virus infects and replicates inside cytoplams of the cell

Phases of T cell response:
1. Naive t cells are recirculating through the peripheral lymphoid organs searching for foreign antigens
2. Antigens are transported from the portals of entry to the lyphoid organs
3. The antigens are recognized by APC's (dendritic cells) and are presented on the APC surface with class 1 and class 2 MHC
4. The APC Starts expressing receptors on its surface as a flag for the T cell to know that it has an antigen (there receptors are for the CCR7 chemokine released by T cells)
- CCR7 is being produced by antigen specific T cells all the time. Once the APCs receptors are produced, the APC will migrate from low CCR7 concentration (far from lymph nodes) towards the area of higher CCR7 concentration (nearest lymph node)

5. Once the T cell binds to the APC, it gets activated
6. The activated T cell starts secreting cytokines which stimulate the proliferation producing more and more antigen specific T cells (clonal expansion)
7. Activated T cells undergo differentiation into T effector cells which can:
- Remain in the lymph nodes and eradicate any infected cells (CD8 function)
- Send signals to B cells and macrophages to promote antibody responses against microbes (CD4)
- The effector cells leave to sites of infection to eradicate infected cells there
- They leave to peripheral sites developing into memory T cells

8. Once the effector T cells eliminate the infectious agent, the stimuli that triggered T cell differentiation and expansion are also eliminated, returning the system to its basal resting state.

END RESULT OF T cell ACTIVATION:

1. Clonal expansion and proliferation
2. Differentiation into T effector cells

1. Recognition and co stimulation:
- Mediated by:
    1. Antigen receptor (TCR) responsible for recognizing the MHC-peptide antigen complex on APCs. Coreceptors CD4 and CD8 strengthen this connection and bond stabilizing the connection bw TCR and MHC
    2. Accessory molecules:
        - Adhesion molecules: LFA-1 Lymphocyte function associated antigen. LFA-1 binds to ICAM-1 on APCs (Intracellular adhesion molecules)
        - Co stimulatory molecules: B7 proteins on the APC is recognized by CD28 receptors on T cells. They give out a costimulatory signal. The signal leads to production of IL-2 by the T helped cell, which fully activates the T cell, producing

T memory cells (PRODUCTION OF IL-2 is the vital step for activated T cell production)

With no costimulatory signal —> **Anergy** specific to that epitope (they still respond to other epitopes). This helps as a defense mechanism against an immune response twds self proteins.

To turn off the helper T cell, CTLA-4 appears on the T cell surface displacing CD28, binding to B7. —> Co stimulatory signal is inhibited —> IL-2 is not produced —> No T cell activation..

- This helps regulate the balance between on and off status of T cell activation.
- NO CTLA4 —> ACTIVATION ALWAYS ON —> Autoimmune reactions
- CTLA4 —> On and off continuous and regulated —> Improves tolerance and prevents autoimmune diseases and rheumatoid arthritis
- CTLA4 AGONISTS: Reduce immunity
- CTLA4 ANTAGONISTS: Increase immunity

Another way to turn off T helper cell activation: PD-1 programmed death protein which interacts with PDL-1 on APC surface. —> immune response is reduced

Effector functions of T cells:
1. regulatory cells (helper) and regulate by signal
2. Effector T cell (perform the function itself)

- We have **two subpopulation** of T cells: **TH1 and TH2** which are responsible for secretion of different interleukins, as they have different functions:

1. **TH1 cells**: induced using IL-12
- By IL-2: Activates other CD4 and CD8 T cells and stimulates CD8 T cells to become activated cytotoxic CD8 T cells
- By IFNγ: Stimulates proliferation of B cells to produce IgG1 and IgG3 secreting plasma cells (IgG1 and IgG3 are effective opsonins), this helps enhance killing by macrophages **< IN DELAYED HYPERSENSITIVITY >**
- Induces cell mediated immunity

2. **TH2 cells**: Induced by IL-4
- By IL-4 and IL-5 it leads to development of B cells into plasma cells that secrete antibodies, especially IgE
- Induces antibody mediated immunity

Differentiation of antigen specific T cells in CD4 or CD8 T cells:

T cell progenitor cells —> Thymic hormones (thymosin and thymopoietin) in outer layer of cortical thymus nurse cells —> T cell subpopulation with significant surface proteins (CD3 CD4 and CD8)

CD3:
- Present on all T cell surfaces
- Associated with TCR receptors
- Five transmembrane proteins (one of them is zeta)4

- Function: Transmission of signal from outside to inside the cell
- Linked to fyn kinase for signal transduction

CD4:
- One transmembrane protein
- Lck kinase for transduction

CD8:
- Two transmembrane proteins
- Lck kinase for transduction

TCR complex:
Consists of the TCR Alpha and TCR beta chains, zeta chain, CD3

Balance between Th1 and Th2:

Th1 Stimulation= IL12 (stimulates Th1) + IFNγ (inhibits Th2)
Th2 Stimulation= IL-4 (stimulates Th2) + IL-10 (inhibits Th1)

Th-17:

- Subpopulation of immune responders that secretes IL-17
- IL-17 is a signal that recruits neutrophils to the site of infection
- Plays an imp role in mucosal immunity of GI tract

Signal Transduction:

1. Antigen- MHC complex on APC interacts with TCR on the surface
2. This produces a stimulatory signal which is transmitted by CD3 to the nucleus
3. This leads to or induces an influx of calcium into the cell
4. This activates Calcineurin enzyme
5. This enzyme switches on the genes for IL-2
6. Once switched on, the IL-2 is produced (Committed step, no turning back)

To block calcineurin function we use **cyclosporine** (useful in organ transplants)

Sheet 19

Immunodeficiency disorders:

Primary:
- Rare
- Genetic: inherited
- Mostly monogenic
- Mild to nearly fatal (the earlier the more severe)
- Either autosomal Dominant or recessive (**mostly recessive**)
- Affects either adaptive or innate responses
- (**most common cause is humoral (B cell)**)

- Clinical signs:
  1. Family history (strongest predictor)
  2. Infection in more than one anatomic location
  3. Increase frequency and severity of disease with age
  4. Recurrent serious infections with common pathogen (respiratory, trachea etc by infection of common bacteria and virus)
  5. Serious infection with unusual pathogens
- Opportunistic pathogen —> Mostly has ID's
- Examples on opp. pathogens: 1. Candida infections, 2. Mycobacteria intracellulare, 3. Pseudomonas respiratory infection, 4. JC virus
- Diagnosis:

1. History (fam history and previous infections)
2. Physical examination (Normal response to infection -> lymph node enlargement) (PID patients -> absent lymph node -> B cell defect)
3. CBC and differential: Know which type is defected by using blood diff tests
4. Quantitate Ig's : Conc of immunoglobulins from highest to lowest in norm: IgG, IgA, IgM, IgD, IgE. All defected -> **B cell defect (most common IgG subclass deficiency is IgG2)**
5. Review prev culture results: know type of organism and severity
6. Titers of administered vaccine: Normally- Immune response (production og IG's)
7. Lymph enumeration
8. Skin testing
9. Measure complement activity: **Most common complement def. (C4).**
10. Phagocyte studies NBT: phagocytosis and respiratory burst defect (No ROS) . Bacteria is engulfed and not killed
11. Enzyme studies: non functional enzymes

important

Disorder	Disease	
	OPPORTUNISTIC INFECTIONS	OTHER SYMPTOMS
Antibody	Sinopulmonary (pyogenic bacteria) Gastrointestinal (enterovirus, giardia)	Autoimmune disease (autoantibodies, inflammatory bowel disease)
Cell-mediated immunity	Pneumonia (pyogenic bacteria, *Pneumocystis carinii*, viruses) Gastrointestinal (viruses), mycoses of skin and mucous membranes (fungi)	
Complement	Sepsis and other blood-borne infections (streptococci, pneumococci, neisseria)	Autoimmune disease (systemic lupus erythematosus, glomerulonephritis)
Phagocytosis	Skin abscesses, reticuloendothelial infections (staphylococci, enteric bacteria, fungi, mycobacteria)	
Regulatory T cells	N/A	Autoimmune disease

PID's Primary immunodeficiencies				
	Cause	What happens	Infections	Treatment
IgA selective deficiency **Most common congenital ID**	- Lack of differentiation of B cells into IgA producing plasma cells	- No IgA detected - Other Ig's are normal	- Genitourinary Tract infections - Respiratory infections (Mucosal surfaces)	

PID's Primary immunodeficiencies				
X Linked Bruton's Agammaglobinemia	(X-linked) Mostly in men. - Deficiency in Bruton Tyrosine Kinase enzyme in B cell progenitor - > Lack of differentiation of pre B cells	1. No plasma or B cells (due to no differentiation) 2. T cells are normal in number 3. Absent adenoid or tonsils 4. Lack of Ig's of all classes 5. Lack of CD19 positive B cells 6. Pre B cells accumulate in bone marrow	1. Sino-pulmonary infections (due to encapsulated bacterial infection - strep, meningococci, Haemophilus influenza) 2. Meningitis 3. Dermatitis 4. Bacterial Otitis Media 5. Bronchitis 6. Pneumonia	- Prophylactic antibiotics and replacement therapy (Passively IV administered Antibodies)
CVID common variable immune deficiency **Largest group of symptomatic PID**	Defect in the differentiation of mature B cell to plasma cells.	- 2 or more classes of IG's levels lower than usual	Respiratory tract infections by common bacteria	
	T cell DEFECTS:			
DiGeorge Anomaly	**Chromosome 22 region q11**		Tetany in neonatal period due to hypothyroidism (leads to hypocalcemia) Cardiac defects	

Sheet 20

Secondary:

- More common than PID's
- Acquired resulting from exposure to external agent
- Reversible if the causal factor is removed
- ex: protein malnutrition (most common ID)
- **Factors** :

1. Malnutrition
2. Stress
3. Drugs
4. Age extremes
5. Surgery and trauma
6. Environmental factors
7. Infectious disease
8. Metabolic diseases

- **Malnutrition**: affects both innate and adaptive immunity
  - Hypoproteinema: Reduced T cell production and function
  - Vitamin D deficiency: Lower macrophage activity against intracellular pathogens (M. Tuberculosis)
  - Micronutrient deficiency: Weakened barrier mucosa —> Increase invasiveness of pathogens

- **Extremes of ages- neonates:**
  - Inverse relationship between susceptibility to opportunistic infections and age of prematurity. (premature are more susceptible than older children)
  - Why? Due to several factors:
    1. Neonates have fewer marginal B cells in lymphoid tissue
    2. Decreased expression of CD21 on B cell —> limit ability of B cells to develop responses
    3. Absence of memory cell development due to maternal environment isolation
    4. Lower NK cell activity
    5. Lower TLR signaling
    6. Lower maturity of secondary lymphoid organs
    7. Decreased neutrophil storage pool
    8. Less chemotaxis, phagocytosis, oxidative burst and adhesion
    9. Less complement components
    10. Decreased cytokine production

- **Elderly:**
  - Lower immune defenses (especially in cellular compartment)
  - Decreased delayed hypersensitivity skin reactions
  - Lower lymphocyte proliferative response to mitogens
  - Increased breakdown of skin and mucosal barriers in innate immunity
  - Metabolic and endocrinological changes —> slower healing process

- **Metabolic disorders (DM and uremia)**

  - **DM**:
    - Hyperglycemia leads to less proliferative response to mitogens
    - Less chemotaxis
    - Less phagocytosis
    - Anergy

  - **Uremia**
    - Affects innate and adaptive immune responses
    - Less chemotaxis in vitro
    - No generation of memory antibody responses

- **Drugs:**
  - Glucocorticoids: reduce tissue damage caused by inflammatory response
  - How?
    1. The drug binds to cytosolic receptors
    2. There receptors are translocated to the nucleus and act as transcription factors

3. They modulate and change the expression of certain genes
4. They lead to anti inflammatory effect
5. Modulates signal transduction
6. Activates transcription factors NFKB, AP1, and Nuclear factor of activated T cells

- **Pulse therapy: (corticosteroid therapy). What it does:**

1. Phagocyte and lymphocyte anergy
2. Lymphopenia due to pro apoptotic activity and inhibition of Il-2 mediated response
3. Decreased cytokine production (IL-1 IL-6 and TNF)
4. Impaired chemotaxis and cell adhesion of leukocytes

- Leads to: **ORAL CANDIDIASIS** AND **HERPES ZOSTER DISEASES**

- **Calcineurin inhibitors:** inhibit calcineurin (enzyme that activates T cells)
- Ex: Tacrolimus and Cyclosporine
- Mechanism against calcineurin:
    1. Bind to cytoplasmic proteins of the immunophilin family
    2. This prevents the interaction of these proteins with calcineurin
    3. This means IL-2 transcription wont be activated
    4. T cells are no longe activated
    5. This helps prevent:
    - organ transplant rejection
    - graft versus host disease
    - Corticosteroid resistant AI disease

- Cytotoxic drugs: manage AI diseases

- Sulfasalazine and hydroxychloroquine: interfere with DNA synthesis
    1. Arrests cell cycle
    2. Induce apoptosis
    3. Inhibit T and B cell proliferation
    4. Inhibit cellular and antibody responses resulting from prev sensitization

-Infectious diseases (HIV) :

- Double stranded RNA virus
- Enveloped
- Lentivirus group
- Tropism for CD4+ expressing cells (T cells, with CXCR4 coreceptor - and macrophages with CCR5 coreceptor)
- Two types: HIV 1 and HIV2 (2 takes longer to develop immune deficiency than 1)
- Genome consists of 3 structural genes (Gag, Pol and Env) and 6 regulatory genes:
    - **Gag**: Split by HIV protease to give capsid p24, capsid p6 and p2. These form the viral particle and stabilize the genome
    - **Pol**: Split to give three enzymes: **reverse transcriptase** (converts viral RNA to DNA) , **integrase** (integrates viral DNA into the host genome to use the host cell mechanism. and **late phase protease** (cleaves the viral protein of the immature virion for it to maturate after budding from the infected cell)
    - **Env**: cleaved to give 2 envelope proteins (glycoprotein 120 and 41). These help binding to the target cell CD4 receptors and CXCR4 and CCR5

- Regulatory genes:
  - **Transcription activator protein:** induces increased transcription of HIV genes
  - **Rev protein**: Allows expression of different HIV genes via mRNA splicing
  - **Negativity factor protein:** Down-regulate CD4 and MHC class1 to escape immune surveillance
  - **Vif:** inhibits the human antiviral enzyme APOBEC3G (responsible for developing gene mutations during viral transcription)
  - **Vpr and Vbu**: Induce intracellular transport of viral proteins for viral particle formation

Pathogenesis of HIV:
1. HIV GP120 binds to CD4 and CXCR5
2. Infected cells migrate to lymph nodes and replicate and infect adjacent cd4+ cells

Primary acute infection (first stage) _ 1-6 weeks

- symptoms: fever, myalgia, headaches
- Major decrease in CD4 T cells, and memory cells in the gut associated lymphoid tissue (GALT)

- Immune activation
- Hyperviremia

- T cell Lymphopenia induced by HIV: How?
  - HIV induced apoptosis
  - Viral cytopathic effect
  - Apoptosis via nonspecific immune activation
  - Cytotoxicity against infected cells
  - Autophagy via lysosomal pathway: induced by HIV envelope protein in uninfected t cells

Clinical latency stage_ 3-20 years

- most patients infections remain asymptomatic until the end of the latent stage
- Increase in several cytokines to determine level or degree of viremia
- Higher viral load —> shorter clinical latency
- Immune system response: (virus overcomes it with its escape strategies)
  - Release HIV neutralizing antibodies
  - Anti HIV antibodies against infected cd4+ cells
- Without HIV drug treatment —> infections with opportunistic organisms —> Immunodeficiency

AIDS:

- Diagnosed when:
1. CD4 t cell count < 200/microliter
2. Presence of AIDS defining condition

- ***Elite controllers (LNTP) :*** Patients who are HIV infected and remain asymptomatic without antiviral therapy and do not have AIDS

- How does this happen?
- due to CCR5 gene mutations (affecting entry of HIV)
- Low number of activated cd8+ t cells
- viral mutations that result in low virulence.

Diagnosis of HIV:
- using assays
- Followed by a confirmatory test (western blot or reverse transcriptase PCR)

Treatment:
- **HAART**: highly active anti retroviral therapy. Combination of three synergistic antiretroviral drugs that suppress viral replication

  - Benefits:
    1. Reduce viremia
    2. Restores normal t cell count
    3. Reduce number of infections

- In adults **(ARV or ANTI HIV) therapy** is recommended when the patients has an AIDS defining illness or when viral load >100,000 copies/ml
- **ARV**:

1. Nucleoside reverse transcriptase inhibitors
2. Non nucleoside reverse transcriptase inhibitors
3. Integrase inhibitors
4. Protease inhibitors
5. Ccr5 inhibitors
6. Cell fusion inhibitors

Drug-induced effects:

1. **IRIS**: Immune reconstitution Inflammatory Syndrome

Severe inflammatory response to existing opportunistic disease after starting **HAART**

- Treatment: corticosteroid therapy

2. **Maculopapular rash and Steven Johnsons Syndrome**

Due to Trimethoprim, sulfamethoxazole, and nevirapine

3. **Multi organ hypersensitivity syndrome**
- Due to **Abacavir** nucleoside reverse transcriptase inhibitor

Treatment: 72 hours of discontinuing the drug

SCID: Most serious and most severe PID

Causes:
1. JAK3 Deficiency
2. Common gamma chain deletion (X Linked), common to receptors for IL-2,4,7,9,15,21
3. Adenosine deaminase deficiency (Adenosine deaminase is responsible for conversion of Adenosine —> Inosine and Deoxyadenosine—> Deoxyinosine

- This leads to:

1) Accumulation of toxic adenosine metabolites: interferes with DNA synthesis and purine metabolism
2) Liver damage
3) Deafness
4) Neurologic symptoms

4. Mutation in recombinase enzyme (RAG 1 and RAG2)
5. Mutation in genes encoding proteins involved in DNA excision-repair pathways during gene rearrangement (Artemis)

Hyper IgM syndrome: (T cell defect)
X linked disorder

Cause: Deficiency in CD40L or CD154 (on T helper cells) which leads to:
- Impaired communication between APC and T cells
- T cells have fewer CD40L expressed (wont bind to APC's such as DCs or B cells)
- T independent B cell response is unaffected
- Normal to high levels of IgM antibodies, hence the name

Defect of neutrophils: Chronic Granulomatous Disease (CGD)

- Occurs due to NADPH oxidase deficiency
- This enzyme is usually present in phagocytes
- It contributes to the respiratory burst against microbes
- It reduces oxygen to superoxide, hypochlorous acid, hydrogen peroxide
- Susceptible to fungal infection and catalase positive bacterial infection

Complement deficiency:

C3 deficiency: Lupus-like encapsulated bacterial infections
C1 esterase inhibitor deficiency: Angioedema
C5 and C9 deficiency: Neisseria infections

Sheet 22

In an abnormal immune system there are two scenarios:
1. Responding normally to an antigen but the response doesnt turn off (Chronic inflammation)
2. Hypersensitivity Reactions (HSR): Excessive and inappropriate immune response

HSR Causes:

1. Response directed towards self antigens and host tissues (Autoimmune)
2. Inadequate control of immune response against microbes and pathogens
3. Immune response against environmental antigen or commensal microbes

Allergy: occurs against harmless antigens (allergens) which are either protein antigens or glycoproteins that have protease properties

HSR types:

Type 1: (immediate response- seconds to minutes)
Allergy that induces release of antibodies that will switch from IgM producing to IgE producing.

Factors affecting class switching:
1. Dose of allergen
2. Route of administration of allergen
3. TH2 cells that are responsible for humoral immunity (cytokines promote IgE production IL-4 IL-5 IL-10 IL-13)

For hypersensitivity to occur, ***sensitization*** of the antigen must take place. How?

1. Antigen enters body for the FIRST time, and this doesnt induce hypersensitivity
2. Antibodies bind to this antigen and class switching occurs (IgM to IgE)
3. IgE will bind to FC epsilon R1 on mast cells
4. This binding induces the formation of granules that contain inflammatory mediators.
5. The cells are now sensitized after the first exposure to the antigen
6. Second exposure to the antigen will lead to hypersensitivity reaction
7. IgE antibodies bound to the sensitized mast cells and antigen cross linkage occurs
8. This causes degranulation
9. The binding of antigens induces intracellular signals to activate LYN and NAP kinase
10. This activates NFkB
11. This causes the release of already formed granules (containing heparin histamine and protease)
12. Their release induces the **early phase hypersensitivity reaction**
13. These mediators induce Localized inflammation
14. Leads to release of Il-1 and TNF-alpha from mast cells
15. These increase expression of ICAM on endothelial cells
16. This encourages the entry of neutrophils, TH2 cells and eosinophils
17. **Late phase hypersensitivity:** IgE and antigen bind to FC epsilon R2 on TH2 cells
18. This allows TH2 cells to produce cytokines IL-4 5 and 13

Allergy manifestation: - related to the tissue mostly affected -

1. Vasodilation
2. Redness (rash)
3. Vasodilation (leads to shock)
4. Mucous secretions

Biological effects of mediators:
1. Histamine: Increase permeability of venules, contraction of intestinal bronchial and arterial smooth muscles
2. Leukotrienes and prostaglandins: Bronchoconstriction, increased vascular permeability, mucus production
3. Cytokines (IL4 5 and 13): recruitment and activation of inflammatory cells

Local allergies: (manifestations depend on location)

1. Allergic Rhino-conjunctivitis (Hay fever)

- Airborne allergies
- Common in the spring
- Symptoms:
- watery exudation of conjunctiva, nasal mucosa and upper respiratory tract
- Coughing and sneezing

2. Atopic dermatitis (eczema)

- associated with filaggrin mutation
- Leads to defective skin barried (tight junctions become loose)

3. Food allergies

-symptoms:
- GI symptoms
- Respiratory symptoms (suggests anaphylaxis which is Ig-E mediated and can be reversed using EpiPen, to help induce vasoconstriction)

4. Asthma:

- Atopic Asthma: IgE-mediated response to allergens like pollens dusts, fumes, etc
- Intrinsic Asthma: triggered by exercise, drugs or cold (dont need allergin to get induced but are still considered HSRs)
- Symptoms:
- Airway edema
- Mucus secretions
- Inflammation
- Bronchial constriction
- Shortness of breath and wheezing

5. Anaphylaxis:
- Systemic
- IgE mediated
- Allergen is directly administered into the blood or through GI (food)

- Leads to anaphylactic shock
- EpiPen: Epinephrine (leads to vasoconstriction to retain the blood)
- Leads to 1. cardiovascular collapse 2. Asphyxiation due to laryngeal edema

Remember: 1st exposer to the allergen in type 1 HSR —> sensitization
2nd exposure (re-exposure) —> cross-linking IgE antibodies with antigens on mast cell surface inducing release of inflammatory mediators

Allergy testing:

- Subcutaneous injection of allergen in different regions, checking for swelling after a short time (immediate reaction)
- Wheel and flare
- Control tests:
  1. Positive histamine test: If the patient isn't sensitive to histamine, this means the results of the test are difficult to interpret
  2. Saline negative test: if the patient is sensitive to saline, then he or she is too sensitive to give correct test results (could lead to false +ve)

Cause of allergy:

1. Atopy (genetic tendency to develop allergy):
- Involves association with:
- cytokines
- chemokines
- HLA type
- IgE production
- FC epsilon RI and RII

2. Exposure to environmental conditions
Both of these together give us hypersensitivity reactions

Management of allergy:
1. Avoiding identifies allergen
2. Topical steroids (anti inflammatory)
3. Antihistamines, B2 adrenergic agonists (bronchodilation), epinephrine
4. Sodium cromoglycate (reduce mast cell degranulation)
5. Desensitization (immunotherapy): It inhibits Th2, and induces Th1 response (humoral) and Induces T reg.

Type 2: (Antibody-mediated) - hours -

Against auto-antigens on our cells and tissues

- Initiated by the interactions between antibodies and Ags on cell membranes and ECM.
- Antigen —> Self (autoimmune disease) or non-self
- Antibodies are specific (organ specific response)
- Localized effect not systemic (unlike type 3)
- Penicillin (antigen in all types of HRS)

- Tissue damage occurs via: 1. Complement activation (ADCC for Nk cells), 2. Phagocytosis
- Diseases:

1. Immune mediated hemolysis:

- Someone who is Rh -ve and is exposed to Rh +ve blood which produce antibodies
- Allo-immunization (pregnancy Rh sensitization): If the mother is Rh-ve and the baby is Rh +ve —> Mother produces IgG antibodies —> First pregnancy isnt affected since antibodies take time to be produced —> Mother is re-exposed to Rh+ve blood in second pregnancy —> Mother's Ab's will bind to baby's RBC's —> hemolysis

2. Goodpasteur's syndrome:

- Antibodies act against collagen 4 in basement membranes of kidneys and lungs
- Manifestations: Hematourea, proteinurea, Pulmonary hemorrhage

3. Pemphigus Vulgaris:

- Antibodies act against the desmoglein in the tight junctions of the skin
- Manifestation: bullae

4. Myasthenia gravis: (type 5)

- AI disease
- Manifestations:

Muscle weakness, ptosis (eyelid drooping), fatigue

5. Graves (type 5)

- Antibodies act against TSH
- Hyperthyroidism

6. Wegeners granulomatosis:

- c-ANCA against proteinase 3 in PMNs
- Manifestation : vasculitis

Type 3: (Immune complex mediated) - hours -

- Against soluble antigens forming immune complexes in circulation or in joints.
- forms inflammatory manifestations
- Systemic
- Excessive formation of immune complexes lead to complement activation-> Inflame
- Diseases:

1. Systemic Lupus Erythematosus:

- Rash (butterfly rash)
- Antibodies against nuclear antigen (DNA)

2. Post strep glomerulonephritis:

- Prognosis: fever

Type 4: (DTH Delayed type hypersensitivity) 48-72 hours

Overstimulation of receptors that the antibodies bind to (example (hyperthyroidism)

Sheet 24:

Melanocytes are triggered by UV light

Causes their transformation to tumor cells —> melanoma —> metastasis to other sites in the body (liver and the lung)

The Immune cells that monitor our tissues:
1. NK cells: They identify stress associated molecules on damaged and cancerous cells
2. Cytotoxic T cells bind to MHC1 on DC's and later on bind to tumor associated antigens
3. Once the T cells and NK cells are activated they release granzymes and perforin
4. They induce apoptosis of the cell
5. T helper cells support this by binding to DC's and helping them activate CT T cells
6. Helper t cells then release IFN gamma, which activates more NK cells

How cancer cells respond to this:

1. Survival advantage: Cancer cells develop mutations that change their antigens. The antigens that were once recognized by the T cells and NK cells are no longer there, due to the cancer cells constant development of mutations. (immuno editing)
2. Protect themselves from the T cells by expressing PD-L1 on their surface, this binds to PD1 on cytotoxic T cells. This pushes the T cell away, before it gets the chance to release perforins and granzymes
3. Tumor cells attract immune cells, that suppress the activity of immune cells (T reg cells) which inhibit the activity of Cytotoxic T cells.

Coleys toxin:
1. Local injection where the tumor cells are
2. The injection contains Weakened bacteria (Toxins that have been exposed to heat)
3. Toxins provoke the immune response

Immune therapy (different strategies) that increase the immune function against tumor cells:
1. Non specific immune stimulation
   - Inject in (vivo) molecules that boost immune response
     1. Activate APC's
     2. APCs recruit T cells
     3. The T cells are further activated —> better response against the tumor cells
   - Ex on molecules injected: (IFNalpha & IL-2)
   - BCG vaccine (originally used to treat TB) - weakened bacteria induced inflammation - used in bladder cancer
2. Adaptive cell transfer:
   1. - Extract immune cells outside of the patient —> specific targeting. we take tumor cells that have immune cells amongst them (the ones interacting with the tumor cells) we then separate the immune cells from the tumor cells and amplify them. Then inject the patient with the amplified immune cells.
   2. - Taking cells from the blood: collect T cells from the blood —> genetic modification —> insert CAR gene —> CAR receptors expressed on T cells —>

inject into patients body —> CAR induces T cell response without the need of MHC

3. Immune-checkpoint blockade:

- (CTLA-4, which bind to B7 on the APC. This inactivates the T cell pushing It away. The cancer cell takes advantage of this.)
  - To solve this we Use (1) neutralizing antibody (Ipilimumab) will neutralize CTLA 4. No interaction between CTLA4 and B7. Another way of blockade is (2) Use neutralizing antibodies that bind to PD1 to stop this molecule from switching off cytotoxic T cell. (PREVENTING PD1 AND PDL1 FROM BINDING TO EACH OTHER)

4. Vaccination:

1. Specific vaccination for a certain antigen. Virus is engineered in a way to weaken it
2. Infect the patient with this virus
3. The body recognizes antigens of the virus and induces specific response against it

1. Use the tumor cell itself (weaken it)
2. Weakened tumor cell antigens are recognised and the body fights against it

- Vaccinate Actual immune cells (APC immature cells). Mix them with antigen of interest —> maturation of APC —> mature APCs are injected —> Stimulates the immune response against the presented antigens.