* **Lec 13 – Generation of Lymphocyte Diversity**

How flow cytometry works: 1. WBCs in a blood sample is labeled with fluorochrome antibodies. 2. Those cells are passed through a laser beam. 3. A detector detects the light wavelength emitted/absorbed by each different fluorochrome antibody. 4. Computer software calculates percentage of each cells type based on their wavelength difference which then displays them on this four quadrants graph with the lower left being double negative = cells are not labeled with antibodies, and top right being double positive = cells that are labeled with both types of antibodies = found only in thymus.

Bare lymphocyte syndrome lacks MHC II which binds to CD4 hence would see fewer cells in the CD4 quadrant on flow cytometry

B and T cell receptors review: TCs bind only linear epitopes vs BCs/Abs bind 3D and linear epitopes. Ag binding site specificity is called V regions. Within the V regions we have hypervariable regions called CDRs with CDR3 being most diverse. Ag receptors constant regions are basically what shapes the effector functions and structure.

What are the steps in lymphocyte maturation? It all starts with proliferation induced by **IL-7** (produced in BM and thymus). Early proliferation = Pro stage = consists of random genetic Ag receptors rearrangement (mostly a failing process = apoptosis) that produces BCs/TCs expressing only one chain of Ag receptor 🡪 Pre stage expresses one chain 🡪 late proliferation = random genetic rearrangement to make the rest of the receptor chains 🡪 immature stage 🡪 Positive and negative selection 🡪 mature stage.

To recap: Pro stage 🡪 Pre stage 🡪 immature stage 🡪 mature stage.

Deficiency in IL-7 🡪 SCID = shutdown in B/T cell immunity. While T cell maturation is completely blocked, BC maturation is intact. Why? We have other growth factors that can do IL-7’s job. Why is it a SCID then? Since BCs require TC help in order to become fully activated. In other words, they are all stuck in Pro stage

What will a deficiency in adenosine deaminase cause? Another form of SCID. Why? Since ADA cleans up toxic purine metabolites (salvage pathway in purine synthesis), its absence will cause…yep, you got it, genius… toxic buildup 🡪 kill developing lymphocytes 🡪 decreased T/B cells.

How are TC’s receptors so diverse? By random genetic rearrangement of various VDJ regions on Chromosome 7 for β chain and VJ regions (no D regions) on chromosome 14 for α chain.

What about Ab rearrangement? Very similar to TCR. With random VDJ rearrangement of the heavy chain which then joins one of the constant regions (Cγ, Cδ) with the Cμ being the first (IgM). Note, random DJ combine first and then V joins DJ 🡪 V-DJ. CDR3 happens at the recombination site between V-D and D-J. If heavy chain recombination is successful, light chain genes recombination ensues. For TC rearrangement, if β chain is successful, α chain recombination follows.

VDJ recombinase is the enzyme that brings the V, D, and J gene segments together and cuts the DNA at specific sites. This enzyme consists of Rag 1 and 2 proteins which is responsible for recognition of DNA signals on VDJ segments. After recognition 🡪 DNA is cut 🡪 double strand breaks 🡪 VDJ recombination 🡪 ligation with recombined VDJ segments.

SCIDs as a result of faulty VDJ recombination: RAG 1 or 2 deficiency = no recombination. ARTEMIS mutation = codes for endonuclease that resolves hairpins during VDJ recombination 🡪 VDJ recombination shuts down 🡪 no B/T cells.

What else adds to the vast diversity of B/T cell receptor besides VDJ recombination (combinatorial diversity)? Additional method of addition/removal of nucleotides at the junctions of V, D and J gene segments = **junctional diversity**. This is accomplished by exonucleases = remove nucleotides and **TdT** enzyme = adds random nucleotides at the junctions.

While the upside of this random recombination is diversity, the downside is that vast majority B/T cells produced by this method are rubbish (like that toddler talking gibberish trying to combine random syllables) and will die by neglect☹.

* **Lec 14 – Lymphocyte maturation and Selection**

What happens if the VDJ recombination of Ig heavy chain (Pro B) does not result in productive rearrangement? It gets a second chance to recombine again the VDJ segments. And if that’s still not productive 🡪 apoptosis.

And if it is successful? Then μ heavy chain protein gets transcribed/translated = Pre B. While most of the heavy chain protein remains in the cytoplasm of the maturing B cell, some of them gets expressed on the cell surface together with a “surrogate light chain” (since the real light chain has not yet been developed) = Pre B cell antigen receptor. Pre B Ag receptor + Bruton’s tyrosine kinase (BTK) = green light signal to continue in development 🡪 rearrange light chain as well as suppress recombination of Ig heavy chain on the other chromosome allele = allelic exclusion. BTK deficiency = no “go ahead” signal 🡪 stall in TC development.

*Clinical Correlation:* Defect in BTK gene 🡪 XLA (X-linked agammaglobulinemia) = BCs are stalled in Pre B stage and low/absent BCs 🡪 recurrent resp. infections and viruses like enteroviruses (Ab dependent immunity).

Light chain will recombine in a similar manner as heavy chain with some minor differences. Light chain only has VJ segments and no D segments to recombine. Light chain can either form a κ or a λ chain with no functional differences. If recombination is not productive, it gets a second attempt and if that’s not successful 🡪 apoptosis. But if it is productive 🡪 light chain will combine with heavy chain to form 🡪 IgM = immature BC. Final maturation occurs in spleen (or in BM) which involves the coexpression of IgD and IgM = mature B cell.

During the BC immature stage it undergoes negative selection = exposed to self-antigen in BM to screen those BCs that bind to self-Ag with high affinity. If it does bind with high affinity 🡪 either reactivate RAG to generate a second light chain and change specificity of the Ag receptor = receptor editing. OR die by apoptosis. All to avoid autoimmunity.

What about maturation of TCs? They’re very similar to the steps in BC maturation. In response to IL-7, stem cells 🡪 pro TC = double negative = expresses neither CD4 nor CD8. VDJ recombination of β chain expressed with pre-Tα chain = pre-TCR complex 🡪 signal for pre TC 🡪 rearrangement of α chain 🡪 immature TC aka double positive = expresses both CD4 and CD8. If the receptor (rearranged by VDJ) binds MHC I (with low affinity , CD4 will drop and CD8 will remain. Similarly, if it binds MHC II, CD8 will drop and CD4 will remain expressed. This is positive selection. And negative selection is double positive that recognize to strongly.

Positive selection = low affinity/avidity recognition of self antigen. Negative selection = those who recognize with high affinity/avidity.

**AIRE** genes in the thymus 🡪 transcription/translation of hundreds tissue specific genes 🡪 presented to developing TCs (e.g. insulin genes) 🡪 TCs that don’t recognize or bind too strongly to those molecules 🡪 removed by positive or negative selection

*Clinical Correlation:* mutation in AIRE 🡪 autoimmune diseases. APS-I, APS-II (autoimmune polyendocrine syndrome) and IPEX. APS-I 🡪 autoimmune Addison’s Disease = autoimmune adrenalitis, autoimmune hypoparathyroidism and chronic mucocutaneous candidiasis are examples of APS-I.

* **Lec 15- T Cell Activation**

What are the steps required for T cell activation? Antigen recognition: TCR recognizing peptide antigen on MHC. Either CD4 with MHC II or CD8 with MHC I will associate TCR and contribute to generation of activating signal. In addition, we have accessory molecules for activation. CD3 complex transmits activation signals into the T cell. Adhesion molecules: integrin **LFA-1** on T cells bind ligand **ICAM-1** on APCs for stabilization = productive response.

Does a single TCR-MHC complex engagement activate TCs? No. At least two or more engagements are required.

Is the integrin-ligand bond high or low affinity? Initially, low affinity but once TCR-MHC bind with right signal, APC releases cytokines which signal integrin-ligand to become high affinity.

What else is need in order to activate the TC? A second signal aka constimulatory signal. APC’s **B7**-1 (CD80) or B7-2 (CD86) binds TC’s CD28 receptor. APC then releases cytokines. Activation of TC in absence of costimulation 🡪 anergy throughout its lifespan.

What is **CTLA-4**? Receptor found on TCs, it binds the B7 ligands on APCs but it serves as suppressors for TC activation by sending inhibitory signals instead of stimulatory. PD-1 is another example of an inhibitory receptor. Loss of CTLA-4 function 🡪 autoimmunity. Blocking CTLA-4 can enhance tumor-specific immune response (inhibition of inhibition).

What is antigen cross presentation, when is it used and what APC is good at it? That’s when an APC presents antigens via MHC I and MHC II (all APCs use MHC II for Ag presentation) and dendritic cells are really good at it. Since CD8 cells need MHC I presentation in order to become activated, APCs use cross presentation for that purpose.

How can CD4 cells help APCs to activate CD8? Either by activating the APC to express high levels of B7 or by producing **IL-2** which promotes CD8 differentiation. Once CD8 is in effector phase (after being activated in LN with help) it does not require costimulation and it can kill non-APCs (that lack costimulation).

How does binding of MHC + costimulation activate the TC? Through the intracellular CD3 region of the TCR which transducer the activation signal. With ITAM (activating motifs) being an important domain of CD3 chains which gets activated when phosphorylated.

How is ITAM phosphorylated? When TCR binds to MHC-peptide complex 🡪 CD4 or CD8 part binds to MHC 🡪 CD4 or CD8 now brings a tyrosine kinase, named Lck, close to ITAM 🡪 Lck phosphorylates all ζ (zeta) chains of CD3 🡪 this becomes docking site for Zap70 (Jones’s favorite molecule/night club trademark) which also gets phosphorylated = active Zap70 🡪 recruits other enzymes and intermediates that ultimately 🡪 formation of transcription factor **AP-1**. OR Zap70 can activate phospholipase C 🡪 ↑ intracellular Ca2+ 🡪 couple more steps which ultimately activates transcription factor **NFAT** or PL-C can activate transcription factor **NFκB**.

In summary: ITAM 🡪 Zap70 🡪 activation of either TF AP-1, NFAT or NFκB 🡪 TC effector mechanisms.

Once activated, how do TCs clonally expand? Via IL2 produced by activated TCs and NFAT is especially important in the induction of TCs to produce IL2. Cyclosporin (used in transplants) blocks IL2 production by inhibiting NFAT.

What is a superantigen? A microbial toxin that binds simultaneously MHC II and TCR in absence of a specific peptide antigen (just brings them together) and causes the TC to become activated. A superantigen is dangerous because it can cause mass activation of TCs 🡪 massive cytokine release 🡪 toxic shock syndrome (septic shock). Staph aureus is an example of a microbe that produces superantigen toxins common in food poisoning.

* **Lec 16 – T Cell Differentiation**

What cells and how does CD4 cells help to get activated? Macs, B cells, DCs. By binding with their CD40-L to CD40 receptor on Macs, BCs and DCs and secreting cytokines.

How does CD4 cells activate macs to kill engulfed microbes and also activate BCs in helminthic response? Via different subsets of CD4 = Th1 and Th2. Another CD4 subset is CD17. **Th1** secrete IFNγ 🡪 activate macs (classical) to be a better killer whereas **Th2** secrete IL4, IL5 and IL13 🡪 activates mast cells, eosinophils, and induces BCs to class switch to IgE 🡪 extracellular helminthic parasites and are involved in allergic diseases. **Th17** secrete IL-17 and IL-22 in response to extracellular bacteria/fungi (recruit mainly neutrophils).

How do naïve T cells differentiate into different subsets? Cytokine baby, cytokines. DCs and macs produce **IL-12** 🡪 CD4 cells to differentiate into Th1 (as well as IFNγ by NK).

What cell is involved in tuberculin skin test (for *M. Tb*)? Th1 which produces IFNγ 🡪 induces inflammation. Th1 normally helps macs to better kill engulfed *M. Tb*.

What other function does Th1 cell have in addition to activating macs? Th1 secrete TNFα 🡪 ↑ expression of adhesion molecules on endothelium as well as producing cytokines to promote inflammation.

What cell is responsible for delayed type hypersensitivity response causing collateral tissue damage? Th1. DTH may occur during a protective response to a microbe or may be autoimmune by nature. Tuberculin skin test is an example of DTH which takes about 48 hours vs. skin allergy test which is an immediate hypersensitivity response and takes minutes to respond.

Which lymphocyte is involved in diabetes type I autoimmunity? Th1 stimulated by some pancreatic β cell antigen 🡪 activates macs 🡪 secretes cytokines that causes cell damage and death. CD8 is also involved by binding MHC I 🡪 induces apoptosis (via Fas-FasL).

What are the different functions of Th2 cytokines? **IL-4** 🡪 B cells class switch to **IgE** 🡪 opsonize helminth 🡪 recruits eosinophils 🡪 binds and degranulates killing the helminth. IgE also sensitize mast cells = involved in allergic reactions. **IL-5** activates **eosinophils** to release major basic protein to kill the helminth. IL-4 and IL-13 🡪 antihelminth in intestines. OR IL-4 and IL-13 turns macs into alternatively activated macs (M2), in absence of strong TLR signals, to suppress immunity and enhance tissue repair (while Th1 releases IFNγ 🡪 classical macs).

What cytokine is responsible for Th2 differentiation? IL-4 which comes from CD4 itself in absence of IL-12 to guide it in the Th1 pathway. (Kinda like in the fetus where female is the default in absence of antimulerian hormone). Or it may come from mast cells and basophils.

What cytokines are responsible for Th17 differentiation? TGFβ, IL-6 and IL-23.

Once a CD4 cell receives the cytokine for subset differentiation, it will inhibit other cytokines from influencing the T cell to differentiate to the other subsets. For example, if IFNγ 🡪 Th1, the other subsets will be inhibited from differentiating to Th2 or Th17.

*Clinical Correlation:* individuals infected with *Mycobacterium leprae* can either have the clinical presentation of **Tuberculoid leprosy**, which have a good infection clearance rate (low infectivity, localized, macs do a better job), or **Lepromatous leprosy**, which have poor clearance (high infectivity, infection spread). What makes these two clinical forms different is their Th response. The former has a dominant Th1 response while the latter form has a Th2 dominant response (mycobacteria grow in macs unabated).

How do CD8 kill? Similar to NKs = perforin-granzyme complex and Fas-FasL interaction.

* **Lec 17 – B Cell Activation**

B cell activation can occur either via T independent (TI) antigens = cross links multiple receptors with bacterial polysaccharides or lipids; or via T dependent (TD) antigens = requires CD4 signal help. CD4 help is required for class switching. Most pathogens antigens are a mix of TI and TD. TI activation class switching is very limited from IgM to IgG2 and of low affinity.

What’s the importance of humoral TI immunity? Defense against encapsulated bacteria (polysaccharide cell wall) and natural antibodies like the ones to A and B blood group glycolipids.

How does TD B cell activation go about? Basically BCs bind to peptide antigen (peptide only since T cells cannot bind anything other than peptides), present it to CD4, CD4 in turn gives BC the proper activating signal, BC can now class switch from IgM, increase affinity and produce memory for subsequent exposures.

Where are TD and TI B cells found? Follicular BCs (found in follicles of LNs) = TD = switch isotypes. Marginal zone (splenic white pulp) B cells as well as B-1 cells in mucosal tissue = TI B cells = do not switch isotypes.

What is the intracellular mechanism of B cell activation? Very similar to T cell activation process. Ag binds 🡪 tyrosine residues of ITAM on Igα and Igβ chains get phophorylated 🡪 phosphorylated tyrosine residues recruit another kinase to phosphorylate tyrosine residues on adaptor proteins 🡪 which recruits downstream signaling molecules 🡪 transcription factor gets activated 🡪 transcribes genes involved in proliferation/differentiation of BC

Do BCs also require co-stimulation in order fully differentiate/proliferate? YES! But costimulation in BCs are a bit different than TC’s B7. One of the costimulation is deposition of C3d (from compliment) on microbial surface. BCR binding to microbe + BC’s complement receptor (CD21) binding to C3d on microbe (costimulation) = strong signal. The other costimulation possible is activation of TLR signal (by pathogens) which synergizes with BCR Ag binding = strong signal. OR let’s not forget of TD activation. CD4 help + BCR binding of Ag (must be protein, of course).

How do CD4 TCs meet, interact and activate BCs? In peripheral LNs, parafollicular TCs express ↑ CCR7, and marginal BCs express ↑ CXCR5. (The ↑ expression of those receptors is what them to the LN and to their specific designated zones, in the first place). Now, in order for BCs to be able to migrate to the TC zone it needs to downregulate the BC zone receptor, CXCR5, (which keeps them there) and upregulate the TC zone receptor, CCR7. The opposite process is required for TCs to migrate towards BC zone = TCs ↓ CCR7 (TC zone receptor) and ↑ CXCR5 (BC zone receptor) 🡪 migrates towards BC zone. Once they meet, BC presents Ag to CD4 via MHC II, CD4’s CD40L binds CD40 on BC 🡪 activation. In addition, CD4 secretes activating cytokines

What determines the BC’s isotype class after receiving CD4 help? The cytokines CD4 cells release after binding to BCs. IFNγ (by Th1) 🡪 IgG1 and IgG3 class switch = best for opsonization of extracellular bacteria/viruses. Transferred through placenta. IL-4 (Th2) 🡪 IgE = anti helminthic immunity and allergy (mast cell degranulation). IL-5 and TGF-β 🡪 IgA = mucosal immunity.

*Clinical Correlation:* X-linked hyper IgM syndrome = defect in CD40L (on T cell) 🡪 cannot class switch = stays IgM forever. These individuals are susceptible to pyogenic bacteria, fungi and intracellular microbes (sinces macs also used CD40L for activation).

On a molecular level, after CD4 signals, how do BCs class switch? Since isotype is characterized by the constant region of the heavy chain, VDJ region doesn’t recombine but remains with the same specificity. So after receiving the class switch signal, upstream switch region of the constant mu (Cμ) genetic region will combine with the downstream switch region of the Cγ region. Since the two switch regions combine skipping all the genes caught in between, namely the Cμ genes. After some splicing, we’re left with the VDJ + Cγ genes 🡪 IgG heavy chain. The same process occurs with the other isotypes.

How do B cells increase its affinity after class switching? Via point mutations in hypervariable regions (CDR3 region) after class switch signal = somatic mutation aka affinity maturation. If BC now binds with lower affinity due to point mutation 🡪 apoptosis. If higher affinity 🡪 selected 🡪 proliferate.

Plasma cells are antibody secreting effector B cells. They go and live in bone marrow, gut or lactating mammary glands

When an antibody binds an Fc receptor (**FcγRIIB**) on BC 🡪 inhibition of B cell receptor signal = used in feedback.

* **Lec 18 – B Cell Effector Functions**

Most effective vaccines today work by stimulating neutralizing antibodies production. Neutralization consists of Ab binding to either microbial surface = inhibits from binding and infecting host tissue/cell, or binding to toxins.

Most effective opsonins = IgG1 and IgG3. What makes them so effective is their Fc regions ability to bind to **FcγRI** on macs and neutrophils. This Ab-neutrophil interaction is the major mechanism of fighting encapsulated bacteria (e.g. pneumococci) and the spleen is the most important site of clearance of those opsonized bacteria hence people without a spleen are susceptible to infections involving those bacteria.

How do NK cells recognize/bind to antibodies? By expressing FcγRIII + binding to Abs Fc regions 🡪 induce apoptosis in that Ab coated cell.

How do eosinohils recognize/kill helminthes? By expressing **FcεRI** + binding to Ab coated helminth 🡪 degranulate

How is IgE involved in allergic reaction? Allergen gets into host tissue, binds to BCR and via Th2 help class switches to IgE, the IgE BC sensitizes mast cells by having mast cell bind to IgE constant heavy chain portion. Next time allergen comes back and binds to IgE 🡪 mast cells degranulate = allergic reaction.

What complement pathway is activated by Abs? Classical pathway. With IgM (or certain IgG) being most effective.

How do BCs class switch to IgA in mucosal tissue? Ag binds to M cells in intestinal lumen 🡪 M cells go back to lamina propia where it presents/causes BCs to class switch to IgA in presence of TGF-β, IL-5 cytokines (in LNs) 🡪 which is programmed to go back to mucosal tissue 🡪 dimeric IgA in lamina propria binds to poly-Ig receptor 🡪 goes through luminal epithelial cells into the lumen = secreted IgA. The poly-Ig receptor staying attached to dimeric IgA is what prevents the IgA from being broken down in intestinal lumen hence can provide passive immunity to nursing infant. IgA plasma cells in breast tissue are what secrets IgA into milk.

*Clinical Correlation:* IgA deficiency = inability of naïve BCs to class switch to IgA = susceptible to respiratory, GI and urogenital infections. But is not an indication for Ig replacement. Why? Since IgA is needed in mucosal tissue and IV Ig replacement will give patient circulating IgA but not mucosal.

How is IgG transferred from mom to fetus across placenta? Via Fc receptor FcRn found in placenta 🡪 passive immunity that has a duration of 3-4 months and is why there’s an ↑ in bacterial infections (e.g. H influenzae) after this period.

Is FcRn only found in placenta? No. It’s also found in adults and is what protects IgG from being degraded = accounts for IgG’s long half-life.

* **Lec 19 – Regional Immunity**

What is regional immunity? Local immunity like MALT and GALT (mucosal).

Where are the majority of Igs found? In mucosal tissue as well as for lymphocytes.

What does the mucosal immunity have to be tolerant of? Various foreign Ags that are not pathogenic as well as for vast amounts of commensal (harmless) bacteria that hang out in the gut.

How does mucosal immunity accomplish this tolerance? By ↓ expression of PRRs to almost non-existent (except for crypts) on the luminal surface of gut epithelium. How is pathogenic microbes recognized then? By intracellular PRRs after being taken up in an endosome.

What are Paneth cells? Specialized epithelial cells found in intestinal crypts, have higher PRRs expression than the rest of epithelium and produce anti-microbial proteins = defensins.

What are the benefits of the commensal gut bacteria? Digesting enzymes that the body does not produce, competitive exclusion keeps pathogenic bacteria out, and helps with immune development.

What is γδ T cell? T cell that has γ and δ chains instead of α and β chains. They don’t recognize MHC associated peptides and their receptor function resembles PRR (innate) more than conservative TCRs.

Where do intestinal effector functions for adaptive immunity occur? GALT (Peyer’s patches, appendix etc) = lymphoid tissue. M cells take up Ag from lumen 🡪 transcytose into underlying lymphoid tissue 🡪DCs take up Ag 🡪 DCs present to naïve TCs/BCs (DCs can also extend arms into lumen and take up Ag independent of M cells) 🡪 TCs/BCs get activated 🡪 travel through blood back to intestines to fight microbe.

What are the two types of dendritic cells (DC)? CD11b = effector DC which stimulates TCs into action. CD103 = regulatory DCs suppresses immunity (e.g. for commensal bacteria) by secreting TGFβ 🡪 naïve TCs into Tregs which suppresses inflammation via IL-10 and TGFβ.

What two TCs are mostly involved in gut immunity? Th17 = extracellular bacteria and fungi. Th2 = anti-parasitic.

How is live-attenuated polio vaccine administered and what immunity is involved? Orally and is IgA dominated immunity.

What is unique to IgA class switching? It can occur via T dependent and T independent mechanisms (low affinity). An exception to the rule that TD help is required in order to class switch.

What is involved in TD IgA class switching? CD40-CD40L binding + TGFβ cytokine

What is involved in TI IgA class switching? TLR activated DCs secrete class switching factors (like TGFβ and other cytokines) although this IgA is of low affinity (whereas TD is high affinity).

What Ags induce oral tolerance vs protective immunity? Food protein and commensal bacteria = oral tolerance since they do not elicit TLR mediated innate response. Invasive bacteria, viruses and toxins = protective immunity since they evoke an innate response.

How do naïve and mature TCs know how to come to mucosal tissue? Naïve TCs = ↑ CCR7 🡪home to peripheral lymphoid organs such as GALT. If activated, TCs change adhesion molecule expression from CCR7 and L-selectin 🡪 α4:β7 integrin and chemokine receptor CCR9 🡪 drain back to blood 🡪 MAdCAM (receptor for α4:β7 in mucosal blood vessels) direct effector TCs back to lamina propia/intestinal epithelium. MAdCAM is expressed in other mucosal tissues as well (e.g. respiratory) = providing systemic immunity.

Where in intestinal mucosa are CD4 vs CD8 found? CD4 are mostly found throughout LP of villus/crypt (inner area) whereas CD8 are mostly found in the epithelium (outer area).

* **Lec 20 – Immune response to pathogens**

\*A very repetitive review lecture from exam I and exam II material\*

Fungi infections are most common in individuals with compromised immune system (e.g. AIDS). Innate immunity against fungi = neuts and macs phagocytose, neuts produce ROS and lysosomal enzymes hence individuals with neutropenia are extremely susceptible to fungal infection. Adaptive immunity = IL-12 🡪Th1 response 🡪 IFNγ + CD40L 🡪 activates macs. CD8 is also involved as well as Th17 (which recruits neuts).

Chronic intracellular fungal infections 🡪 granuloma formation 🡪 impairs tissue function (but walls off fungi).

How do some fungi evade immune response? By blocking cytokine production such as TNF, IL-12 and promotes IL-10 production 🡪 Th2 response (less effective).

Innate immunity against viral infections: PRRs recognize viral RNA/DNA 🡪 production of type I IFNs (IFNα and β) = inhibits viral replication. Also, activation of NK cells 🡪 destroys virally infected cells.

Adaptive immunity against viral infections: either humoral = Abs bind virus early in pre-invasion stage 🡪 prevent their cellular entry (e.g. IgA in mucosa). Or CD8 response post invasion stage = infected cells.

How do some viruses evade immune response? Cellular immunity by inactivation of TAP transporter, removal of MHC class I molecules from the ER (avoid CD8 but prone to NKs), blockade of proteosome, create decoy class I molecules (avoid NKs), infect CD4 (HIV). Humoral by producing IL-10 🡪 Th2 response.

Innate immunity against parasites (macs, complement) is not very effective. Cellular immunity varies with different parasites and stages. Th1, CD8 and Th2 are involved with different helminthes. Some eggs are just walled off 🡪 granuloma. Parasitic invasion varies and are similar to the ones seen with viruses.

* **Lec 21 – Memory and Vaccines**

Primary humoral immune response is dominated by IgM whereas secondary exposure by IgG.

During secondary response, memory BC response suppresses naïve BC response. How? Recall FcγRIIB binding 🡪 inhibitory signals to BCs.

Markers to differentiate naïve TCs vs effector and memory TCs: Naïve = CD45RA, Effector/memory = CD45RO.

How are memory TCs maintained in the body post exposure? Cytokines baby, cytokines! CD4 require IL-7 to keep them around for future exposures to the same Ag while CD8 require IL-7 and IL-15.

Two subsets of memory TCs: **effector memory** TCs express ↓ L-selectin (CD-62L) and CCR7 so they’re going to LNs but straight to peripheral tissue. **Central memory** TCs express ↑ of CCR7 and L-selectin 🡪 go to LNs to gain effector functions but they will proliferate rapidly (vs naïve) and generate more effector cells. So effector memory TCs are fastest to respond.

Preventative vaccination goals are to induce Ag specific memory B and T cells to respond quickly should the individual become exposed to the real pathogen.

Two types of immunization: **Passive** = administration of Igs. Used for non-immunized post exposure when individual is at risk of developing disease or immunodeficient person. **Active** = administration of Ag to evoke a primary immune response so memory cells are produced for subsequent exposure (mostly Ab mediated).

How to measure serum levels of Ag-specific-Ab: indirect ELISA = Ag is inserted into well, patients serum is added and if it has Ab for that Ag will bind Ag 🡪 second Ab is added that binds to the patients Ag bound Ab 🡪 second Ab has an enzyme conjugated to it which can react with substrate added 🡪 change of color.

What are adjuvants? They’re added to vaccinations to cause a stronger immune response = increase immunogenicity. Alum is most common adjuvant found in U.S. vaccinations.

* **Lec 22 – Central/Peripheral Tolerance & autoimmunity**

Central tolerance: negative selection for those TCs that recognize displayed self-antigen with high affinity 🡪 either apoptosis or TCs can try to recombine VDJ. AIRE gene is involved in displaying tissue specific Ag.

Peripheral tolerance: when TC recognizes self Ag in peripheral tissue 🡪 anergy (via CTLA-4 or PD-1 binding to B7 on APCs = inhibition), death, or suppression by Tregs (CD4 and constitutively expressed CD25 = can inhibit TC activation or effector functions via IL-10 and TGFβ production 🡪 inhibits macs and DCs).

**IPEX syndrome** = mutation in FOXP3 gene 🡪 absent or dysfunctional Tregs. BMT early on can prolong survival

Defects in activated induced cell death (AICD) 🡪 autoimmune syndromes like autoimmune lymphoproliferative syndrome = caused by signaling defects of Fas.

Central B cell tolerance: similar to TCs, high avidity self antigen recognition 🡪 receptor editing or apoptosis. Low aviditiy 🡪 anergy.

Many autoimmune diseases are linked to particular MHC alleles although that’s not the only factor.

Molecular mimicry: cross reactive TC that recognizes microbial Ag as well as self-Ag (e.g. rheumatic fever).

* **Lec 23 – Transplantation & Tumor Immunology**

Two types of tumor antigens: tumor specific = not present on any other cells except cancer. Tumor associated = expressed by tumor but also on certain normal cells (reactivation of embryonic genes or overexpression of normal self-protein

CD8 cells are principal mechanism in tumor immunity, but require APCs and CD4 help for activation. CD8 cells kill via induction of apoptosis (granzyme/perforin complex pathway or FasL-Fas pathway)

How can tumors avoid immune recognition? Loss of MHC or TAP (type I IFN possible treatment), loss of co-stimulation, antigenic variation, some tumors produces immunosuppressive factors like TGFβ, IL-10 (e.g. melanomas, multiple myeloma), difficulty of penetration of solid tumors by TCs, exhaustion of TCs, recruit Tregs = suppress anti-tumor responses.

Autologous graft = transplant within the same individual. Syngeneic graft = transplant between two genetically identical individuals. Allogeneic graft = transplant within same species but genetically different individuals. Xenogeneic = between different species. Alloantigens = molecules on allograft recognized as foreign (by alloreactive lymphocytes). Xenoantigens = on xenografts.

In tissue transplantation we worry about MHC matching but not in blood transfusion, why? RBCs don’t have MHC.

Four types of blood types: A, B, AB and O. A has A antigens and B Ab. The opposite is true for B. But AB has both A and B Ags hence no Abs = universal acceptor. O types have no Ags hence have AB Abs = universal donors. These Abs are naturally occurring and requires no presensitization. Whereas RhD Ags do require presensitization first in order to produce anti-RhD Abs (RhD- receiving blood from RhD+ individuals) hence O- is universal donor and AB+ can receive blood from anyone.

Types of solid graft rejection: **hyperacute rejection** = minutes to hours (can occur even before surgeon is even done sewing up the patient). **Acute rejection** = about 10 days and is focused on the parenchyma. **Chronic rejection** = months to years and is focused around the vasculature (causes occlusion).

Hyperacute rejection can occur when receiving organ from different blood type donor. E.g. type O recipient receives kidney from type A donor. Anti-A Abs in recipient’s blood bind to A Ags expressed on endothelial cells of kidney’s blood vessels 🡪 neutrophils recruitment 🡪 tissue destruction. OR HLA-I on vascular endothelium can also cause hyperacute response if recipient has been exposed previously to those MHC molecules. Cross match test before transplantation. But most organ transplants are performed across some HLA I/II differences.

**Direct** pathway of allorecognition = APCs from donor’s kidney goes to recipient’s draining LNs 🡪 activates them 🡪 TCs go back to kidney and starts to attack donor tissue. In other words, in recognizes foreign MHC molecules (on donor APCs) as if it’s their own = cross reactivity. This damaging mechanism via type IV hypersensitivity.

**Indirect** pathway of allorecognition = when recipient APCs take up/process/present donor’s Ags to CD4 cells via MHC II while direct pathway can involve CD4 and CD8. Acute and chronic rejection uses this pathway.

One way of measuring donor-recipient compatibility is performing mixed lymphocyte reaction (MLR). This works by taking donor’s mitotically inactivated cells and mix with recipient’s lymphocytes and look if there’s a response (by adding radioactive nucleotides and if lymphocytes respond, it’ll need to divide/replicate its DNA and use these radioactive nucleotides which is measured).

The three most important MHC matching for transplants are: HLA-A, HLA-B and HLA-DR.

* **Lec 24 – Hypersensitivity Reactions**

**Type I** (immediate) hypersensitivity = IgE-mast cell mediated via strong Th2 response (↑ IL-4) to harmless environmental Ags. Mast cell binds to Fc portion of IgE Ab via Fcε receptor and when IgE binds an allergen 🡪 mast cell degranulates.

Under expression of **T-bet** transcription factor (for Th1 differentiation) and over expression of **GATA-3** (for Th2)

The first exposure to an allergen = sensitization 🡪 BC activation to class switch to IgE (Th2 help). The allergic reactions will begin to occur in the subsequent exposures. So before allergic response can occur, the person must be sensitized to the allergen (first exposure does not cause allergic response but from second and on).

Once mast cells are activated 🡪 degranulate. Histamine is one of the substances released and can bind to receptors H1, H2, H3. Binding to H1 (found on SM and endothelial cells) = acute allergic reaction = ↑ vessel permeability (causing edema and inflammation), SM contraction (in airways) and ↑ mucus secretion on mucosal epithelial linings

Leukotrienes (also from mast cells) = similar activity to histamine but 100x more potent. Prostaglandin D2 🡪 ↑ BV dilation and permeability, and chemoattractant for neuts.

The immediate reaction is mediated by rapid release of histamine (vasodilator) = wheal (in “wheal and flare” reaction) and slower-acting cytokines (TNF, IL-5) = flare = accumulation of RBCs within minutes.

Types of allergic response depends on route of exposure/entry. I.V. mediated exposure 🡪 systemic mast cell activation = shock. Allergic rhinitis 🡪 nasal mucosal exposure. Oral exposure 🡪 either systemic or local.

**Type II** (antibody mediated) hypersensitivity = Ab binds to tissue Ag 🡪 complement activation 🡪 recruit neuts (via C5a, C3a) 🡪 tissue injury. Or opsonization (C3b, Abs) 🡪 phagocytosis (e.g. RBCs).

Another effect of Ab binding tissue Ag is leading to abnormal physiologic responses without tissue injury. E.g. Grave’s disease = Ab against TSH receptors on thyroid epithelial cells 🡪 mimics the action of TSH = stimulates thyroid epithelial cell to produce/release of thyroid hormones (T3, T4) 🡪 hyperthyroidism. Another example: myasthenia gravis = Abs bind ACh receptors on muscle = blocks receptor from binding ACh = muscle atrophy.

Penicillin allergy is an example of Type II = penicillin (the hapten) combines with carrier on RBC surface 🡪 hapten-carrier complex recognized by Abs 🡪 complement/opsonization 🡪 lysis or phagocytosis.

Goodpasture’s disease is another example = Abs bind to type IV collagen in basement membranes of kidney and lung 🡪 complement 🡪 recruit neuts 🡪 release inflammatory mediators 🡪 basement membrane destruction 🡪 coughing and urinating blood (glomerlulonephritis).

**Type III** (immune-complex mediated) hypersensitivity = similar to type II except Abs bind to soluble Ag 🡪 forms immune complexes (IC) and will only cause type III hypersensitivity if they get deposited in tissues. Once deposited 🡪 activation of complement 🡪 recruits neuts 🡪 inflammatory response. Clinical manifestation all depends where the ICs get deposited (e.g. in small BVs = vasculitis, in renal glomeruli = nephritis, in joint spaces = arthritis, if inhaled = farmer’s lung, in subcutaneous = Arthus reaction).

Arthus reaction = ICs deposit in subcutaneous tissue 🡪 complement activation 🡪 C5a causes mast cell degranulation (esp histamine) 🡪 ↑ BVs permeability, inflammatory mediators 🡪 local inflammation, swelling, phagocytosis, BV occlusion = hard swelling.

SLE (lupus) = Abs binding DNA 🡪 ICs 🡪 nephritis, vasculitis, arthritis.

Polyarteritis nodosa = Abs against HepB virus surface Ag 🡪 vasculitis.

Poststreptococcal glomerulonephritis = Abs against streptococcal cell wall Ag 🡪 nephritis

***\*Both Type II and Type III depend upon complement activation\****

**Type IV** (T cell-mediated) hypersensitivity = can be mediated through CD4 (Th1 or Th17 subsets secreting cytokines) or CD8 killing MHC-I associated Ags.

Th1 secrete IFNγ 🡪 macs + inflammation. Th17 secrete IL-17/IL-22 🡪 neuts 🡪 inflammation + tissue injury.

DTH (discussed earlier) is a type IV Th1 mediated (activates macs). Tuberculin skin test is an example of DTH. Injected Tb Ags are picked up by macs which present it to sensitized CD4 cells (sensitized is what makes the results positive) and memory Th1 cells recognizes and produce IFNγ 🡪 activates macs 🡪 inflammatory cytokines which recruits mediators 🡪 positive Tb test. *Candida* Ags are used to test TC function. Loss of DTH response to those Ags is an indication of deficient TC function. Granuloma = chronic DTH response.

Allergic contact dermatitis (ACD) = reaction to specific environmental Ags like poison ivy, poison oak. But person must first become exposed (sensitized). First exposure = sensitization phase = no reaction. Second exposure (and on) = elicitation phase = full reaction. The Ags are hapten which binds to self-protein carrier (langerhans cell membrane) 🡪 hapten-carrier complex 🡪 presented to TCs in draining LNs 🡪 TCs responds 🡪 epithelial injury.

Example of CD8 mediated reaction is in virally infected cells (viral hepatitis). CD8 show up to kill 🡪 cell damage