# Janeway's Immunobiology - Notes

## Contents

# Innate Immunity

# Induced Response of Innate Immunity

Innate immunity consist of (molecular) pattern recognition receptors:

- free receptors in serum (ficolins and histatins (complement))
- membrane bound phagocytic
- membrane bound signaling
- cytoplasmic signaling

## Phagocytes are a diverse family of cells that kill microbes

### Many phagocytic cells

*Macrophages* come from:

- Progenitor cells that enter various tissues *in the fetal state* and produce macrophages throughout life of organism (!)
- Monocytes circulate freely throughout blood or crawl endothelium

Found in especially large numbers in connective tissues: gastrointenstinal submucosal layer, lungs (bronchi, alveoli), spleen (where they remove senescent cells).

Examples include *microglial cells* in neurons and *Kupffer cells* in the liver, stimulated by IL-34 in both.

*Monocytes* come in two forms:

"Classic" monocytes, that differentiate into active monocytes and macrophages, circulating freely in blood. They make up **90%** and express CD14 only.

"Patrolling" monocytes that "crawl" along endothelium. They express both CD14 and CD16. Do not differentiate.

*Granulocytes* include:

- Neutrophil
- Eosinophil
- Basophil

*Dendritic cells*:

- cDCs (classical) digest and expose microbes as antigen material for the adaptive immune system (the bridge between active and innate immunity)
- pDCs (plasmacytoid) produce type I interferon

### Phagocytosis

Phagosome fuses with lysosome to form phagolysosome.

Some chemistry terminology:

- A radical is an atom, molecule, ion that has at least one unpaired valence electron
- Red(uction)Ox(idation) reactions are thsoe that transfer electrons between species
- Reactive Oxygen Species (ROS) are:
  - Superoxide ( (O_2)- )
  - Nitric Oxide is an example of a radical

Nitric oxide and superoxide are produced in the phagolysosome to neutralize microbes.

### Phagocytic Receptors

- C-type lectin-like receptors (eg. Dectin 1)
- Mannose Receptor (now believed to clear endogenous carbs that are released during inflammation)
- Scavenger receptors (Some bind to low density lipoproteins (see notes on "foam cells" here)
- Complement / Fc receptors (connection to complement system and active immune system respectively)

### G-Protein-Coupled Receptors

Class of ancient receptors that stimulate intercellular killing.

- fMet-Leu-Phe (fMLF) receptor (fMet initiates peptides in bacteria)
- C5a receptor (cleaved C5 molecule from activated complement system)

The receptor has 7 membrane spanning regions and *associate* with (do not "have") G-proteins - heterotrimeric units. When a ligand binds to the receptor, it swaps a GDP with a GTP, and the G-protein splits into alpha and beta-gamma blocks.

These subunits interact with *Rho family GTPase proteins*, Rac and Rho, that activate microbicidal behavior. These induce, among other things, the production of Reactive Oxygen Species (ROS).

*NADPH oxidase*, also called phagocytic oxidase, produces superoxide (O_2-) when fully assembled, but requires recruitment of cytosolic components to membrane components. The cell then can use NADPH oxidase in a process called respiratory burst, to consume lots of oxygen and produce ROS. Neutrophils using lots of respiratory burst die and create pus.

Genetic defficiency of NADPH cause release of less reactive oxygen species. Those with CGD are more prone to bacterial/fungal diseases.

Neutrophils can also release their nuclear chromatin as web like traps called NETs (nuetrophil extracellular trap) to immobolize microbes for phagocytosis (!).

#### Inflammation

Cytokines + chemokines facilitate inflammation.

Four kinds of modification to blood vessels:

- Vessels dialate
- Endothelial cells express adhesion factors to trap circulating leukocytes. (Leukocyte migration is called *extravasation*)
- *Endothelial activation* - Endothelial cells become permissive and blood flows into tissue (edema)
- Clotting

What causes these inflammatory responses? Few things:

- Lipid mediators of inflammation (prostaglandins, leukotrienes, PAF) produced by degraded membranes
- chemokines + cytokines eg. TNF-alpha
- C5a from complement

If there is wounding, the *kinin cascade* and *coagulation cascade* are also spun up that regulate blood pressure, pain, coagulation.

### Toll-like receptors

Broad class of PRR (pattern recognition receptors)

Toll was a receptor initially discovered to control dorso-ventral (front-back) patterning in fruit fly embryo. It was later discovered by Jules Hoffman that the same receptors released antimicrobial peptiedes.

Important PAMPs:

- lipoteichoic acid (gram positive)
- lipoprotein (gram negative)
- flagellin
- unmethylated DNA

#### TLR Structure

Each TLR has 20-25 Leucine Rich Repeats (LRR) that form a convex hook. They sometimes form dimers with eachother.

Binding a ligand causes TLR to dimerize or causes a conformation change with a preformed dimer.

All mamallian TLRs have an interleukin receptor in their tail. (Not really sure the role of this. Assume its involved in sigaling cascade)

**TLR 1 / 2 / 6** Among other ligands recognized are:

- lipoteichoic acid (gram negative)
- Triacyl + diacyl lipoprotein (gram negative)

These guys dimerize (1 <> 2; 2 <> 6)

**TLR 5** Bind to a conserved (intact) flaggelin domain that is deep within flagella

**TLR 11 / 12** Bind to whole proteins (many unknown) from protozoans and microbes. Expressed in mice.

**TLR 10** Ligand unknown. Expressed in humans

# Antigen Recognition by Lymphocytes

# Antigen Recognition by B-cell and T-cell receptors

**T cell antigen recognition**

**TCRab ~= Fab antibody fragment** Each chain is two Ig domains and they are linked by a disulfite bond

They have a variable and constant domain with homology to the immunoglobulin analogues from antibodies. Cysteine stalk with a disulfite bond.

CDR loops are roughly the same as in antibodies. (Ex: of difference - CDR2 in beta chain is at a different angle)

There is HV4

**TCR recognizes peptide:MHC complex** Recognize short continuous peptide sequences presented from MHC

**Two types of MHCs with different structures** I

- Three domains from alpha chain and one beta_2 microglobulin
- Alpha_1 and alpha_2 form the peptide binding cleft
- Only alpha chain spans the membrane

II

- two membrane spanning alpha and beta chains
- ends of the peptide binding cleft are more open

**Peptides stabilize MHC molecules** MHC are only stable when bound to peptides and this makes their presence on the surface of a cell a reliable indication of infection.

**MHC 1 bind short peptides (8-10 aa)** Peptide fragments are locked in place by their free amino and carboxyl to the peptide binding groove.

(Synthetic peptides lacking these free ends are unable to bind)

MHC 1 is highly polymorphic and allelic variation presents itself along the peptide binding cleft

The key amino acids that contribute to affinity along the groove are called *anchor residues*.

**MHC 2 bind unconstrained peptides** Bound peptides >= 13 aa. MHC 2 proteins lack the conserved regions at the ends of the cleft that bind the peptides in place.

Also have same anchror resiudes amongst families of sequences that bind

**peptide:MHC:TCR complexes have a unique structure** The entire TCR is rotated slightly when it binds to MHCs.

Valpha is roughly on top of amino terminal while Vbeta is roughly on top of carboxyl terminal

Induces some amount of conformational change of receptor - "moving the CDR loops" to slot into grooves of the peptide / cleft structure.

**CD4 / CD8 molecules are TCR coreceptors** Bind simultaneously with the TCR / MHC peptide groove.

CD4 is expressed on "helper" Tcells. (MHC 2) CD8 is expressed on "cytotoxic" Tcells. (MHC 1)

CD4 is a chain of 4 immunoglobulin domains and binds to MHC2 at the hydrophobic cleft formed by the beta_2 and alpha_2 domain

CD8 is a disulfide linked pair of chains (alpha / beta) with a long, glycosylated polypeptide chain attaching it to the membrane. (The glycosylation is thought to maintain its conformation and keep it from getting digested).

Binds more weakly to the alpha_3 domain of MHC1 and the binding affinity is effected by the amount of glycosylation.

**MHC cell type expression distribution** MHCII expressing cells are usually themselves the immune cells that are modulated by CD4+ T cells.

eg. B cells that will change antibody isotype or macrophages that become "activated"

MHCIIs are expressed in:

- B cells
- Dendritic
- Macrophages
- Neutrophils

Most tissues, including the above, express MHC I. Including kidney, liver, etc.

Neither are expressed on non-nucleated (eg. blood cells)

**gamma/delta TCRs are unique**

**Misc. Questions** Why is CDR3 the loop with highest variability?

Has the greatest contact with the peptide during the peptide:MHC:TCR binding. Also has greater combinatorial diversity by the inclusion of the D region during somatic hypermutation.

Why is Fc crystalizable but Fab not?

Overview of the crystallography process?

- Purification
- Construction of a "crystal" - repeated lattice structure
- Crystal growth
- Crystal mounted on a goniometer in front of an xray source

Why did camelids evolve heavy chains?

extreme environments:

- thermal stability
- less resources to manufacture

What are the tx benefits of heavy chains only?

- Smaller / easier to traffic to tissues
- More stable
- Cheaper to manufacture and easier to engineer
- Less immunogenic (look less like a different human's antibody?)

Specific examples of antibody <> epitope interactions for each of the bond types.

# Generation of Antigen Receptors

# The Generation of Lymphocyte Antigen Receptors

### TCR genes are organized in a similar pattern and rearranged by the same enzymes as B cells

The variable domain for the alpha chain is formed from V and J regions. The beta chain is formed from V, D and J regions.

Rearrangement occurs in the thymus. The rearrangement process is the same (RSS regions are homologous and the recombinases, including RAGs, are the same). This means that diseases effecting chain diversity are the same for B and T cells.

*T cell excision loop* is the bit excised

Feature more P / N nucleotides

### TCRs concentrate diversity in CDR3

This is because they bind to the MHC (in contrast to antibodies that bind to much broader range of antigens). CDR1/CDR2 are along the edges of contact surface and bind to the less variable MHC while CDR3 binds to the antigen

There are many more J genes than in immunoglobulin light chains, which directly impact diversity of CDR3.

### gamma / delta chains admit similar structure

Delta chain genes live entirely between V_alpha and J_alpha locus

Delta chains can have two D domains which obviously increases combinatorial diversity but also increases P-N nucleotide insertion zones from one to three (V-D, D-D, D-J).

### Different classes of immunoglobulins are distinguished by heavy chain structure

- IgM (first to be expressed after B cell activation)
- A, G, E, D

IgM + IgG activate C1 in the complement cascade

### Constant regions have distinct functions

- Fc receptor binding
  - IgG receptor macrophages / neutrophils
  - IgE receptor on mast cells, eosinophils

- Complement activation (bind to C1q protein)
- Active transport by engaging *neonatal Fc receptor* (FcRn)

**IgM / IgD are expressed from the same pre-mRNA transcript**

Constant genes lie in 200kb locus

IgM / IgD come from alternatively spliced trnascripts that have not udnergone calss switching.

It has been known for some time that IgM is expressed in immature and IgD in mature.

ZFP318 expression seems to induce the switch from M to M + D, but it is unclear why.

**IgD marks B cell maturation**

**Transmembrane and secreted heavy chains are formed from alternative splicing**

There are two polyadenylation site, before and after the hydrophobic transmembrane domains (M1 / M2)

Recall polyadenylation occurs before splicing! If we cleave at the polyadenylation site before M1 / M2, we get a secreted peptide. If we cleave afterwards, we splice out the hydrophillic domain, leaving M1/M2, and we get a membrane bound peptide.

Activated B cell heavy chains are generally modified to produce the secreted form

**J chains help IgM and IgA bind to each other**

IgM pentamers and IgA dimers are stabilized by an additional 15kD polypeptide chain called a *J chain*.

Repetitive epitopes are common antigens for multimer antibodies. The individual affinity in these cases is often lower, especially because eg. IgM is produced earlier in the affinity maturation process (?), but the avidity or sum of binding interactions is high because there are 5 or 6 antibodies.

**Questions**

- Why do hairpins form with nicks in the RAG recombinase cutting

# Antigen Presentation

**Antigen presentation functions both in arming effector T cells and triggering effector functions**

Cytotoxic T cells (CD8) engage with antigen presenting MHCI from the cytosol and kill cells

Effector T cells (CD4) engage with antigen presenting MHCII in endocytic vessicles.

Cytotoxic:

- Direct presentation - cytosol -> ER -> MHCI
- Cross presentation - take up from other cells

Effector:

- Some effectors, eg. TH_1 cells, activate macrophages to kill pathogens replicating within them.
- Autophagy - ingest pathogens from cytosol into

**Peptides are generated from ubiquitinated proteins in the cytosol by the**

Proteasome - multicatalytic protease:

- 20S catalytic core
- 2 19S regulatory caps

One cap shuttles proteins into the core and the other cap keeps proteins from leaving before they are degraded.

*UPS system* ubiquitination

lysine (target protein | ubiquitin) <> carboxy terminus of ubiquitin

This is assumed to be the main mechanism by which proteins are degraded for MHCI molecules

There is constitutive expression of proteasome core beta proteins, but:

- immunoproteasome have beta proteins induced by interferon
- thymoproteasome have beta protein crucial for CD8 T cell development

Defective Ribosomal Products (DRiPs)

- improper splicing
- translation of a frameshift
- improperly folded Tagged with ubiquitin and degraded

**Peptides from cytosol are transported into the ER by TAP to bind to MHCI**

TAP are ATP-dependent peptide transporter spanning ER membrane (live in the MHC locus) Transports peptides between 8-16 aa in length

**MHCI molecules chill in ER until they bind peptides**

*Calnexin* is a chaperone protein that associated with alpha chain (as well as MHCII, TCR, IGX)

When a beta_2 binds to alpha, the complex disassociates from Calnexin and binds to another group of proteins to form *Protein Loading Complex* (PLC).

PLC:

- calreticulin
- tapasin - bridges with TAP to await a peptide
- ERp57

What is *peptide editing*?

MHCI that are never associated with peptides are eventually ejected from the ER with the ERAD pathway (not specific to MHC-peptide association)

**Dendritic cells use cross presentation**

What if a virus never infects a non-dendritic cell? (Strange question because I'm sure that the)

**Peptide:MHC II generated from acidified endocytic vesicles**

MHC II present peptides generated from vessicles from dendritic cells, macrophages, B cells and present to CD4 T Cells.

MHC II molecules (like all membrane proteins) are deposited in ER and transported to membrane in ER buds, fusing with intracellular vessicles with antigens in them.

Peptides are ingested in different ways:

- receptor mediated endocytosis by B cell surface immunoglobulin

- macropinocytosis (not triggered by binding of cargo)

endosomes become increasingly acidic as they traverse towards the vesicles with MHCII

"acid proteases" play dominant role in antigen processing (are active 2-5 pH)

- cathepsins
- asparagine endopeptidase
- IFN-gamma induced lysosomal thiol reductase Redundant and non-specific (chop things up without much precision)

**The invariant chain directs MHC II to vesicles with peptides**

Ii (invariant chain)

Winds through ER membrane and forms trimers with the inner head.

NH+3 < ER > CO-

CLIP (class II Associated Invariant Chain Peptide) Binds to the peptide binding groove

Trafficking of membrane proteins is associated with cytosolic sort tags, so the Ii ,

**CLIP needs to be displaced from the MHC II**

HLA-DM looks a lot like MHC-II and is found in the endosomal compartment. It binds to MHCII and causes it to change conformation and release peptide

HLA-DM also binds and rebinds to new peptide:MHC II complexes. This will get rid of peptides that are unstably bound. This is good because they need to last for days before interaction with a T cell.

HLA-DO is another molecule that looks like MHC II but is a negative regulator of HLA-DM

HLA-DM is to MHCII as tapasin is to MHC I

**MARCH-1 stops antigen processing in dendritic cells during infection**

MARCH (membrane associated ring finger) is an E3 ligase that ubiquinates cytoplasmic tail of MHC II

Constitutively expressed in B cells, dendritic cells, macrophages (the trinity that express MHC II).

During infection the pathway is shutdown

Also ubituinate CD86, a T cell co-stim.

## MHC complex and function

**Genetic structure of MHC**

Class I

- HLA-A
- HLA-B
- HLA-C

Class II

- HLA-DP
- HLA-DR
- HLA-DQ

Non-classical MHC (Class Ib)

Interferon alpha, beta and gamma all increase transcription of MHC class I genes and associated chaperone proteins for antigen presentation.

Interferon gamma increases the MHC class II and chaperone proteins (including HLA-DM + HLA-DO that bind / help fix antigens in final processing steps)

### Protein products of MHC class I / II are highly polymorphic

**Polymorphism** - across species, number of alleles in a gene.

Some MHC genes have > 1000 alleles.

- Polygenic - multiple copies of gene on single chromosome
- Polymorphic - lots of options for alleles. Increases likelihood of heterozygous genotype and therefore double the number of different MHC proteins
- Codominant - express proteins from both sister chromosomes

The higher frequency of point (vs. silent mutations) than would occur by random chance points is evidence of selection for polymorphism

### MHC polymorphism effects (1) antigen binding and (2) TCR binding

MHC isoforms have very high sequence divergence (20 amino acids) compared to other polymorphic genes

Most of this variability is in the peptide binding groove, effecting the anchor residues of classes of bindable peptides

If you do not have an appropriate MHC you cannot respond to the antigen. This is common in inbred individuals because they are homozygous for MHC genes

*MHC restriction* is the dependence of the TCR on *both* the antigen and the MHC complex when binding.

**Peter Doherty + Rolf Zinkernagel** TCR only kill cells infected by specific virus (obvious) But will not kill cells infected by same virus but different MHC from that which primed it

## Alloreactive T cell

Allogeneic T cells react to non self. Discovered from early mixed lymphocyte reactions. Pool two populations together and irradiate one so they cannot divide. 1-10% of all T cells will respond to cells from another individual in this way.

Alloreaction / alloreactivity - stems from recognition of allelic polymorphism

Two hypotheses:

- Positive selection produces TCRs that bind to *some* MHC and increases likelihood that same TCR cross reacts to another MHC
- Inherit property of TCR to bind to MHC (animals that lack MHC I / II and cannot positively

## T cells respond to superantigens

Minor lymphocyte stimulating antigens (MLs) were found to stimulate T cells but were actually retroviral proteins stably integrated into mouse genome.

The response is useful to the pathogen not the host. It is not specific to the pathogen, so it binds a large number of T cells and causes massive production of cytokines by CD4 T cells - (1) systemic toxicity and suppression of further adaptive immune response.

(eg. toxic shock syndrome from staphylococcal bacteria)

### Polymorphism allows diverse antigen binding

Why not more than 3/4 genes? Autoreactivity.

Self peptides that can bind vs. pathogen peptides that can bind.

# Lymphocyte Receptor Signaling

# Lymphocyte Receptor Signaling

## General principals of signal transduction and propogation

#### Multiprotein signaling complexes

Composition of multiple proteins is crucial for proper downstream signal propogation.

SH2

- YXXZ

Scaffolds Adapters

#### Small G Proteins are molecular switches

Also called **small GTPase**

Include:

- Ras
- Rho
- Rac

Have two states - bound to GDP or GTP.

Role of following in "swtiching" G proteins:

- Guanine Nucleotide Exchange Factors (GNEFs)
- GTPase Activating Proteins (GAPs) are cofactors that

Mostly found in their inactive (GDP-bound) state

Localized to membrane by fatty acids attached post translationally

#### Signaling proteins are recruited to the membrane in different ways

1. Phosphorylation of receptor itself or scaffold, followed by recognition by SH2-domain containing signaling proteins or adaptors
2. Binding to small GTPases already bound to membrane by attached lipids
3. Recognition of locally phosphorylated membrane lipids

*phospholipid phosphatidylinositol* are phosphorylated by enzymes called *phosphatidylinositol kinases* to produce PIP_2 and PIP_3 (2 and 3 phosphates on carbon rings). These enzymes are recruited by its SH2 domain to phosphotyrosine on the tail of some receptor.

#### Post translational protein modification can effect signal response

Phophate and ubiquitin additions to proteins can modulate constituents of signal cascades.

Ubiquitin ligases (like Cbl) can be recruited by SH2 domains, so tyrosine-phosphorylated targets can be modified by additional ubiquitin and then targeted to degradation pathways.

### Antigen receptor signaling in lymphocytes

Antigen binding is not enough - signal needs to transduce to intracellular components...

#### CD3 + ITAMs

Immunoreceptor Tyrosine-based Activation Motifs

## B and T Cell Development

### Development of B Lymphocytes

#### Lymphocytes

HSC

There is a (somewhat continuous) lineage of differentiation.

HSC -> MPP -> CLP -> pro B-cell

MPP - Multi Potent Progenitor, (myeloid + lymphoid only) CLP - Common Lymphoid Progenitor, committed to NK / T / B

Bone marrow stromal cells secrete key cytokines and present ligands necessary to differentiate HSCs into B cells (FLT3). The stromal cells actually remain in contact with developing B cells for much of the lifecycle.

**Lymphocytes derive from HSC in the bone marrow**

**B-cell development begins with heavy chanin rearrangement**

**pre-B-cell receptor tests for production of a complete heavy chain** signals transition from pro B-cell to pre B-cell

**pre-B-cell receptor signaling further inhibits heavy chain recombination** is responsible for allelic exclusion

**Pre B-cells rearrange the light chain and express surface immunoglobulin**

**Immature B-cells are tested for autoreactivity before they leave the bone marrow**

**Lymphocytes that encounter self antigens in the periphery are eliminated / inactivated**

**Immature B-cells arriving in the spleen often die and require positive signals for maturation / long term survival** Become follicular B-cells + marginal B-cells

#### Development of T Lymphocytes

**T-cell progenitors originate in bone marrow but all important development events occur in the thymus**

**Commitment to the T-cell lineage occurs in the thymus following Notch signaling** Major transcription factors from Notch signaling:

TCF1 / GATA3 -> CD3 + Rag3

**T-cell precursors proliferate extensively in the thymus but most die there**

**Successive stages in thymocyte development are marked by changes in surface molecules**

**Thymocytes at distinct developmental stages are found in different parts of the thymus**

**Alpha/beta and gamma/delta T cells arise from a common progenitor**

**Successful synthesis of beta chain allows the production of a pre-TCR that triggers cell proliferation + blocks further beta chain rearrangement**

**T-cells undergo successive alpha chain rearrangement until positive selection or cell death**

**Positive and Negative Selection of T cells**

Prior to alpha/beta receptor expression, development is independent of peptide:MHC interaction.

**Only thymocytes whose receptors interact with self peptide:MHC complexes survive**

**Positive selection coordinates expression of CD4 or CD8 with the specificity of the receptor**

**Thymic cortical epithelial cells mediate positive selection of developing thymocytes**

**T cells that react strongly with self antigens are deleted in the thymus**

**The strength of the signals for positive and negative selection must differ**

# T Cell Mediated Immunity

# T Cell Mediated Immunity

Primary lymphoid tissues - bone marrow + thymus. Secondary lymphoid tissues - lymph nodes, spleen, tonsils, mucous membranes (bowels/intenstine)

Naive T cells circulate between blood and lymphatic tissue.

CD4 Effector Subsets include TH_1, TH_2, TH_17, TH_FH + T_reg.

*Priming* (to distinguish from productive response)

- recognition of MHC:peptide
- binding to costims
- stimulation by cytokines to lead down particular effector path

## Descriptions of Lymphoid Tissues

***Spleen* captures antigens from the blood stream.**

- Central arteriole brings blood to the spleen.
- Branching parts of arteriole end in the marginal sinus.
- Marginal sinus separates arteriole from white pulp
- White pulp is the lymphoid compartment:
- B cell zone
- T cell zone wraps the arteriole on the outside of the marginal sinus (Periarteriol lymphoid sheath)

*Lymph Nodes*

- connected to both blood and lymphatic system

- enter from blood in *HEVs* (High Endothelial Venules)

*Mucosa Associated Lymphoid Tissue* (MALT)

B cells and T cells are seperated into distinct zones in all three lymphoid tissue types. Line epithelial tissues.

**The Development of Secondary Lymphoid Tissues is controlled by TNF proteins + Lymphoid Tissue Inducer Cells**

Lymphoid Tissue inducer Cells migrate from the bone marrow (where they are derived from HSPCs).

They express TNF family proteins - LT_\alpha + LT_\beta. These chemokines cause stromal cells at the precurso sites of 2nd lymphoid tissue, eg. Peyer's patches, to expresss TNFRs.

This causes stromal cells to express CXCL13, which recruits more lymphoid cells from the marrow.

**Chemokines direct T and B cells into their respective compartments**

CCL21, CCL19 are the chemokines that attract T cells. CCL7, receptor expressed by T cells (and dendritic cells), responds to ˆ

When they are recruited, the T-cells themselves start to express these chemokines

CXCL13 (B Cell chemokine)

**Naive T Cells Migrate thorugh Secondary Lymphoid Tissue**

Clonally expand + differentiate into effector and memory cells with antigen specificity. Lymph ti

**Entry of Lymphocytes into Lymphoid tissues requires concerted interaction of chemokines + integrins**

1. Rolling
2. Activation
3. Adhesion
4. Diapedesis (enter)

Selectin is a protein largely conserved with a different carbohydrate recognition motif.

- P-sellectin (macrophages)
- E-selectin (vascular endothelium)
- L-selectin (naive T cells)

Binds to vascular adressins (loose term for class of "adressing" molecules) found on the HEV - CD34 + GlyCAM-1.

**Activation of integrins by chemokines is responsible for the entry of lymphocytes into lymphoid tissue**

Properties of integrins:

- Composed of alpha and beta chain.
- Subfamilies are defined beta chains.
- Bind tightly to ligands after receiving signals that cause changes in their conformation

T-cell integrin or *LFA-1* (Leukocyte Family Antigen 1). Binds to ICAM-[1-3]. Is activated by proximity from selecting rolling

CCL21 bound to the HEV (the same that direct them to the correct compartment) causes integrins (like LFA-1 to change conformation) when bound to . Once bound to ICAM, the lymphocyte migrates into the lymphoid tissue.

**T-cell responses are initiated in the secondary lymphoid organs by activated dendritic cells**

Dendritic cells can be activated by:

- TLRs or other pathogen recognition receptors
- Tissue damage
- Cytokines from infllamation

Activated DCs migrate to the lymph and express costims + antigens needed for TCR binding.

Macrophages + B cells also present antigens. Distribution in tissue differs. Type of interaction with T cell also differs:

- DCs initiate first response for clonal expansion + differentiation
- B cells + macophages mostly present soluble antigens + intracellular pathogen antigens. Recruit primed CD4 T cells.

**Dendritic cells process antigens from a wide array of pathogens**

Dendritic cells originate from myeloid progenitors in bone marrow. There are two types:

- conventional
- plasmacytoid
- Are abundant in barrier tissue sites
- complement receptors
- Fc receptors
- C-type lectins
- general uptake of fluid with macropinocytosis
- Phagocytosis (II)
- Macropinocytosis (II)
- Virus (I)
- Cross presentation from phago/macropino (I)
- Sharing (eg. Herpes Simplex) (II)

**Microbe induced TLR signaling induces migration to lymphoid tissues + kicks off antigen processing**

Activation is also called **licensing**

Different receptors provide maturation signals:

- TLRs
- dual effector function of phagocytosis receptors
- lectin

Then produces CLL7, so it will migrate to CCL21/CCL19 producing cells in secondary lymphoid tissues

Once in there

- High levels of MHC1/MHC2
- Co-stim CD80 / CD86
- Cease ability to perform phagocytosis or macropinocytosis

The activation of co-stims is believed to distinguish self-protein presentation from pathogen-protein presentation. The former does not activate costims. Molecules that do are called *adjuvant*.

#### Plasmacytoid DCs help T-cells but aren't relaly invo

Help conventional dendritic cells produce IL-12, which is needed by CD4 T-cells for production of IF-\gamma.

B cells and macrophages seem to use antigen presentation to enhance *their* own responses

- surface immunoglobulin with antigen <> primed CD4 T-cell -> antibody secreting B cell
-

Macrophage production of MHC I/II + B7 seems to be far more for maintainence + functioniong of effector / memory T cells than any priming. Induced by recognition of MAMP

Main function in lymph tissues is actually ingestion of stray pathogens / apoptotic lymphocytes to keep them from entering the blood.

## Priming of naive T cells by pathogen activated dendritic cells

#### Cell adhesion molecules facilitate initial interaction of T cells with dendritic cells

LFA / CD2 <> ICAM1/ICAM2 When T-cell recognizes an MHC:peptide complex, LFA changes its conformation to bind more stably to ICAM.

#### Multiple signals are involved with the activation + proliferation of naive T-cells

- Activation (MHC:peptide)
- Survival + Expansion (Co-stims)
- Differentiation (Cytokines)

B7 family ligase (acting on CD28) is the common costim molecule. Binding with antigen:MHC is not enough to "prime".

#### Antigen presenting cells deliver multiple signals for the clonal expansion and differentiation of naive T-cells

Useful to consider three types of signals necessary for T-cell differentiation.

1. MHC:peptide <> TCR
2. Costim
3. Effector cytokines (more later)

#### CD28 co-stimulation of activated T-cells induce IL-2 and IL-2R

Costims kick T-cell into G1 and induce expression of the missing \gamma chain from IL-2R and the expression of the cytokine IL-2 itself.

T_reg cells express a more sensitive form of IL-2, which is one mechanism for suppressin of T-cell activity

Recall, IL-2 is induced in 3 ways from costim:

1. AP-1, NFAT from PI-3 Kinase activation downstream of ITAM
2. Increase longevity of cytoplasm transcript
3. PI-3 Kinase leads to Akt which prolongs the life of the cell

#### Additional co-stim pathways are involved in T-cell activation

After activation, additional costim receptors are expressed that are important to know:

- B7 costims <> CD28 related receptors - ICOS, CTLA-4
- TNF receptor family. Generally all activate NFkB (CD40, CD27, 4-1BB)

### Proliferating naive T cells differentiate into effector T cells that do not require costims to act

4-5 days of cell division transform into cells with specific responses upon recognition of antigen.

Best seen by CD8, cytotoxic, cells that should not require CD8 present on surface of eg. cell infected with a virus

Change repertoir of expressed surface molecules

- Reduce L-selectin -> no longer recirculate through lymph nodes
- Express P/E-selectin on surface of vascular endothelium -> migrate from bloodstream to sites of infection

### CD8 T cells are often activated by CD4 T cells

CD8 effects are strong and require more costim than CD4 for priming. CD4 T cells often bind to APC and induce expression of B7 + 41BB-L to facilitate CD8 development. They need "extra help".

### CD4 T cells differentiate into different types of effector cells

Simply memorizing the names, effector molecules, recruited immune cells and function is highest leverage here.

T_H_1. IFN-\gamma. Recruit macrophages. Kill internal bacteria like mycobacterium (Tuberculosis) T_H_2. IL-4/IL-6 (bone marrow) + IL-13 (goblet cell). Recruit basophils, eosionophils, mast cells. Kill Helminth parasites (tapeworms) T_H_17. IL-17 (stroma). IL-22 (epithelial). Recruit neutrophils. Kill extracellular bacteria like pneumoniae. T_FH. IL-21. Mostly involved in isotype switching in B-cells. T_reg. Promotes tolerance to antigens in other T-cells.

### Cytokines induce the differentiation of naive CD4 T cells down distinct effector pathways

Similarly, we shall memorize the cytokines that commit primed T cells to different effector lineages.

These cytokines effect different members of the JAK/STAT pathway. Pattern of JAK/STAT activation leads to unique transcription factor + gene expression program for each effector type.

T_H_1. IFN-\gamma + IL-12. STAT1 + STAT4. T-bet. T_H_2. IL-4. STAT6. GATA-3. T_H_17. IL-6/IL-23. STAT3. ROR\gamma T_FH. IL-6. STAT3. Bcl-6. T_reg. IL-2. STAT5. **FoxP3**

### CD4 T-cell subsets can cross regulate each other's differentiation through the cytokines they produce

Cytokines produced from some effectors can inhibit other effectors.

IL-4 inhibits T_H_1. IFN-\gamma inhibits T_H_2.

### Regulatory CD4 T cells (T_regs) are involved in controlling adaptive immune responses

Can be created in the thymus or in the periphery.

Expression of FoxP3 is the classic marker. CTLA-4 / CD25 are surface markers.

## General properties of effector T cells and their cytokines

### Effector T-cell interactions with target cells are initiated by antigen-nonspecific cell-adehsion molecules

Antigen non-specific interaction of surface proteins LFA-1 / CD2 (which are expressed 2/4x higher than naive T-cells.

**An immunological synapse forms between effector T cells and their targets to regulate signaling and direct the release of effector molecules**

Effector T-cells and target cells form an **immunological synapse** / SMAC (supramolecular activation complex).

There is sometimes an inner and outer zone. The outer zone is where the useful immune activity occurs and the inner zone is where recycling of TCRs via ubiquitin taggin occurs.

Polarization is reorientation of internal organelles to direct effector molecules to a precise area:

- cortical actin cytoskeleton (at site of contact)
- MTOC
- Golgi apparatus

**The effector functions of T cells are effected by the array of effector molecules they produce**

Cytotoxins stored in cytotoxic granules Effector cytokines expressed de-novo

**Cytokines can act locally or at a distance**

TODO: table

**T cells express several TNF-family cytokines as trimeric proteins that are usually associated on the cell surface**

# Humoral Immune Response

# Humoral Immunity

Antibodies contribute to immunity through three key mechanisms:

- neutralization
- opinization
- complement activation

There are three main ideas here:

1. Affinity maturation + class switching are the core programs executed by B-cells, facilitated by T-cells
2. Distribution and function of antibody types
3. How antibodies engage various effector functions

## B-cell activation by antigens + helper T-cells

**Activation of B-cells by antigen involves signals from the B-cell receptor**

T_FH -> CD40L CD40 -> B-cell marker

non-canonical NfKb -> main pathway for proliferation + differentiation ()

**Linked recognition of antigen by T-cells and B-cells promote anitgen**

CD21 / CD19 / CD81

B-cell coreceptor complex CD19 CD21 CD81

response

**B cells that encounter their antigens migrate towards the boundaries between B cell and T cell zones**

It is remarkable that both these cells can be specific for the same type of antigen when the odds are 1/10000.

**Localization of cells is controlled by recptors and chemokines:** T-cells - CCR7 -> CCL19 + CCL21 expressed by stromal + dendritic cells in the *T-cell zones*

B-cells - CXCR% -> CXCL13 expressed by specialized follicular dendritic cell in the *primary lymphoid follicles / B-cell zones*.

B-cells encounter BAFF in the follicles, an important TNF-family cytokine that acts through BAFF-R, but also BCMA + TACI, to activate the non-canonical NFkB pathway similar to CD40.

Antigens are trapped by complement receptors on macrophages in specialized zones (subcapsular sinus in lymph nodes + marginal sinus in spleen). These macrophages do not swallow them for some reason. B cells shuttle between sinus zones and follicle, bringing antigens to the FDCs there.

Movements:

- B-cells first position in the primary follicles for 6-24 hours with EBI2 expression.
- They then express CCR7 and move towards the interface of follicles + T-cell zones where they encounter T-cells for crosslinking.

**T cells express surface molcules and cytokines that activate B-cells that in turn promote T_FH development**

T-cells (early FH linaeage subtypes) express:

- IL-21 -> STAT3 activation in B-cell
- CD30L -> B-cell activation + proliferation
- CD40L -> B-cell activation and proliferation

Prolong and stable physical contact mediated primarily by SLAM (IgG superfamily protein expressed on both cells)

Some reciprocal signaling from B-cells - ICOSL binds to ICOS and leads to T_FH differentiation. ICOS also involved in expressing Bcl-6 and c-Maf

**Activated B-cells differentiate into antibody secreting plasmablasts and plasma cells**

2-3 days after initial activation, B-cells downregulate CCR7 and once again express EBI2 to move into follicles. There they form clumps called the *primary focus*.

The primary focus is mostly made up of plasmablasts. Not all T-cells become plasmablasts in the primary focus and some become long-lived plasma cells. These foci are pronounced 5 days into the infection but are temporary.

*Both types* - Large volumes of antibody secretion. Up to 20% of protein synthesized by cell. Continued expression of surface IG + MHC-II (though less of both in plasma cells).

Then main difference seems to be plasmablasts continued ablity to class switch, proliferate + (potentially) continued somatic hypermutation.

Plasma cells also seem to negatively supress T_FH cells by decreasing Bcl-6 and IL-21 with cognate antigens on MHC-II.

**The 2nd phase of B-cell response occurs when B-cells migrate into primary follicles and form germinal centers**

***Summary***

- B cells become activated by both BCR and MHC:peptide recognition by helper T-cells
- These cell types first
- Major proteins:
  - CD40L -> CD40.
  - ICOS
  - IL-21
- T-cell activation in the germinal center induces proliferation and then differentiation into either plasma cells or memory cells
- The "germinal center reaction" uses somatic hypermutation and class switching to . Somatic hypermutation through AID is unique to B-cells.

## Distribution and function of immunoglobulin classes

Diffussion / transport is a key property of antibody constant chains that determines their function. (Moving across organ barriers from site of synthesis.)

**Antibodies of distinct classes operate in different places with different effector functions**

**Polymeric immunoglobulin binds to the Fc regions of IgM and IgA and transports them across epithelial barriers**

**The neonatal Fc receptor carries IgG agross the placenta and prevents excretion from the body**

**High affinity IgG + IgA can neutralize toxins and block the infectivity of viruses + bacteria**

**Antibody:antigen complexes activate the classic complement pathway by binding to C1q**

**Complement and immune receptors contribute to the removal of immune complexes from circulation**

## The distributions and functions of immunoglobulin classes

**Antibodies of different classes operate in distinct places**

IgM are produced first

IgG is primary class in blood. IgA is primary in secretions / epithelial tissue. Neutralizing over opinization.

IgE binds avidly to mast cells, that release chemicals that cause vomiting, sneezing and coughing.

**pIgR transport IgA and IgM across epithelial barriers**

Generated right below epithelial layer and transported through epithelial cells to surface (eg. the gut or bronchi).

- Synthesized in *lamina propria*
- Internalized by epithelial cells along basolateral surface (both basal + lateral)
- Passes through cytoplasm in a process called **transcytosis**
- Extracellular domain of pIgR + antibody is cleaved

Cleaved EC domain is called **Secretory Component**.

- Bind to mucins in mucus (heavily glycosylated proteins)
- Protect antibodies from cleavage in gut

Where does this happen?

- Gut
- Respiratory epithelium
- Lactating Breast
- Other exocrine glands like tear + salivary

**Gut**

**Bronchi**

**neonatal Fc**

**IgG / IgA neutralize toxins and block infectivity of viruses + bacteria**

Toxins are effective at small concentrations. IgG need to diffuse rapidly and bind with high avidity. Usually a toxic domain and a cell binding domain..

- Clostridium tetani. Blocks inhibitory neuron activiy and leads to chronic contraction.
- Gas gangrene. Clostridium perfringens. Phospholipase
- Diphtheria. Corynebacterium diphtheria. Blocks protein synthesis
- Vibrio cholerae. cholera toxin. elevates cAMP and causes epithelial cells to lose water
- Anthrax. Bacillus anthracis.
- Botulism. Blocks release of acetylcholine, leading to paralysis. Clostridium botulinum
- Whooping cough. Bortedella pertussis. Pertussis toxin -

**Antibody:antigen complexes activate classical complement pathway by binding C1q**

Recall complement has:

- antibody dependent - **classical** pathway - C1q
- antibody independent - **lectin** pathway

The structure of C1q requires that antibodies are bound to the surface of a cell and are not just floating around in serum.

Requires two or more Fc regions. One IgM molecule is enough. 2+ IgG needed. IgM is more efficient at activating complement.

IgM shitfs from planar to staple conformation upon binding antigen and new geometry is suitable for C1q binding.

**Complement + Fc receptors are involved in clearing immune complexes from circulation**

- Fc receptors on phagocytes can clear complexes
- Erythrocytes can shuttle intact immune complexes to spleen and liver. There they are cleared by phagocytes with both Fc + CR1 receptors. Saturation of complex with complement proteins (C4b + C3b) seems to be involved in the recognition + shuttling of the complex.

Complexes that are not cleared deposit on the basement membranes of blood vessels, notably the glomerulus, where podocytes (the cells that filter blood) have CR1 and are responsible in some capacity for filtering

**Summary**

- IgM is the first isotype to express

## Destruction of antibody coated pathogens with Fc receptors

Effector cell recruitment mediated by different Fc receptors is important to neutralize pathogens that are not already neutralized by binding - via phagocytosis or release of cytokines.

Two main components:

- \alpha chain - responsible for binding
- \gamma chain - responsible for signal transduction and transport to cell

**The Fc receptors**

CD64 - IgG - macrophage / neutrophil / eosinophil CD32 - IgG - MNE + B cell / mast cell (can be stimulatory or inhibitory depending on internal domain) CD89 - IgA - macrophage / neutrophil / eosinophil

Some receptors that bear the same name (CD32) are inhibitory and other are not.

Inhibitory Tyrosine associated Immune Motif

TRIM21 is a soluble Fc receptor in the cytoplasm with the highest affinity for IgG. Responsible for clearing ingested complexes by E3 ligation.

**Fc receptors on phagocytes allow ingestion of pathogens**

Some bacteria have capsular polysaccharide on membranes that prevent engulfment and are only susceptible when coated by complement.

These are TI-2 antigens (do not require T-cell help, which includes affinity maturation and memory B-cell). Induce secretion of IgM which recruit compelemnt.

Because immune cell response is inflammatory want to avoid false positives. Require multiple antibodies to be bound to pathogen to avoid this.

Fc receptors result in engulfment (acidic phagosome -> phygolysosome) or release of toxic chemicals.

Macrophages and neutrophils mainly engulf - bacteria / virus. Eosinophils mainly release chemicals - Helminth parasites / worms

**Fc receptors activate NK cells**

Lymphoid lineage cells that have non-specific response:

- Recognize surface proteins directly
- Recognize antibodies bound to surface proteins. This has a name - ADCC.

**Mast cells bind IgE via very high affinity Fc\epsilon receptors**

Fc\epsilon bind with very low concentrations and bind without the presence of antigen. Most circulating IgE are already bound to mast cells in tissue and circulating basophils

After crosslinking of surface-bound IgE by multivalent antigens, fast response:

- release cytokines
- release lipids eg. prostglandin (restrict airway, cause inflammation, recruit immune cells)
- histomines to increase vascular permeability

**IgE important for clearing parasites**

Mast cells:

- recruit immune cells
- create blood flow to traffic antigens to lymph tissue
- muscle contraction expels antigens

Also involved in clearing parasites by clumping in the intenstine - **mastocytosis**.

Eosinophils release granules and are responsible for killing many types of parasites

### Summary

- Antibodies are produced by B lymphocytes and neutralize antigens in different ways - binding, effector cells or complement.
- Antibody production requires a cognate T-cell with similar antigen recognition. B-cells move to T-cell boundary for this.
- Class switching and somatic hypermutation occur in germinal centers.
- B-cells can become plasmablasts?
- T_H produce cytokines that help with class switching

# Innate and Adaptive Integration

# Integration of innate and adaptive immunity

## The distinct phases of infection

innate sensor cells:

- epithelial
- tissue resident mast
- dendritic
- macrophage
- obligate intracellular
- facultative intracellular
- focus of infection

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luminal

### basal

All three of these layers are called the mucosal layer:

- epithelial
- laminal propria {house immune cells}
- smooth muscle

Present in organ systems respiratory, digestive, urogenital.

Epithelial activation -> innate cells traffic through post-capillary venules -> inflammation from cells leads to more cells + leaky epithelial cells provide complement

general, non-specific cytokine response (eg. TNF) specific cytokine distribution from sensor cells dependent on molecular pattern of pathogen

ILCs:

- recruit monocytes and granulocytes
- differentiate specific T-cell effector types

Innate immune system keeps pathogen under control until the active has time to step in

#### Different effector "compartments" for different pathogens

ILCs, effector T, B -> myelomonocytes

myelomonocytes:

- neutrophils
- monocytes
- eosinophils
- basophils

#### ILC1

*Intracellular pathogens*

*IL-12 / IL-18*

_ ILC1 respond to IL-12 / IL-18. Mostly produced by macrophages.

- NK cells are grouped with ILC1. Considered innate and part of this intermediate response?

*IFN-\gamma*

_ IgG _ Macrophages, respond to IFN-\gamma, for cell killing _ T_H1, respond to IFN-\gamma

#### ILC2

TSLP and IL-33/IL-25

*multi-cellular / Helminth parasites*

- ILC2

*IL-5*

- goblet cells in epithelium to produce mucus
- IgE

*IL-13*

- Eosinophils, basophils, mast

Unclear how ILC2 recruit T_H2, but likely through IL-4 produced by eosinophil et. al

- T_H2

#### ILC3

IL-23 IL-1\beta

Activate ILC3, releasing IL-17/IL-22, with following effect on environment:

IL-17, pro-inflammatory, releasing further IL-1\beta, IL-6 IL-22, induces antimicrobrial peptides

### Effector T-cells

#### Changes in adhesion molecule and chemokine receptor expression

T_FH:

- up CXCR5 (home to follicles / B-cell zones)
- down CCR7 (directs T-cells to T-cell zones)
- down S1PR1

Other effectors:

- up S1PR1
- down CCR7

sphingosine 1-phosphate

S1PR1 -> sphingosine 1-phosphate CCR7

L-selectin glycosylated -> PSLG-1

# Mucosal Immune System

# Mucosal Immune System

Primitive organized lymph tissue and antibody producing cells first found in gut of cartilaginous fish Thymus and (bursa of Fabricus? avian) derive from embryonic gut.

## Structure

Most properties of mucosal systems are shared and gut is representative example.

### What is it

75% of all lymphocytes

- gastrointestinal
- respiratory
- urogenital
- middle ear?
- glands:
  - conjuctivae (layer in front of eye)
  - lacrimal (weeping)
  - lactating breast

Distinct features:

- Diffuse lymph tissue and organized structures eg. Peyer's patches
- Specialized antigen uptake cells (M cells)
- Constant inflammatory state / active T cells
- Inhibitory macrophages + dendritic cells
- IgA
- celiaca (response to gluten)
- Chron's (response to commensal bacteria)

### Organization of lymph tissue

Diffuse and concentrated. Lamina propria + GALT

GALT:

- Peyer's patches
- isolated lymph follices
- appendix
- palantine + lingual tonsil, adenoid

GALT + mesenteric lymph nodes make up organized lymph tissue

Peyer's patches (100-200 of them) are formed in the fetal gut. They are lymph follices very rich in B-cells. The epithelial layer between them and gut has a special Microfold cell (M-cell).

Thousands of isolated lymphoid follicles

Small intenstine tissue drain to *mesenteric lymph node* (largest in the body).

**How antigens are sampled in Peyer's patches**

Recall M-cells lack mucus and many pathogens pass through them (*transcytosis*). Dendritic cells + macrophages are waiting on the basal side. Why? CCL20/CCL9 binding to CCR6 + CCR1

1/ Transcytosis 2/ Lymphocyte sampling 3/ Migration from dome to T-cell area

**There are lots of active immune cells in the gut**

*Epithelial*:

- Inter-epithelial lymphocytes. Mostly CD8 T cells "slotted" in the lining.
- \alpha_E:\beta_7 (binds to E-selectin)

*Lamina propria*:

- Range of lymphocytes - CD4/CD8, plasma, macro, dendro, mast, ILCs

Expressed in both. \alpha_4:\beta_7 -> binds to MadCAM on endothelium of blood vessels lining mucosal tissues

**Homing**

Recall CCL19 + CCL21 pull naive T-cells via CCR7 CXCL13 pull B-cells via CXCR5

CCL25 is specific for small intenstine Likely similar for other components, but data unclear

Sometimes primed lymphocytes return to a very specific section of mucosal system. Sometimes they do not, sharing immunity ("common mucosal immune system").

**Dendritic cells**

Two main types in Peyer's patches

CD11b+ - IL-23 ILC3 CD11b- - IL-12 ILC1

Also large circulation of dendritic cells from lamina propria to lymph (eg. mesenteric) where they create effector T-cells. Under non-pathogenic conditions, these effector T-cells regulate the immune response, mediated by retinoic acid produced by D. cells (metabolized from vitamin A).

TGF-\beta produced by D. cells in intenstinal tissues also important for later differentiation

Factors that promote anti-inflammatory behavior of DCs:

- TSL (thymic stromal lymphopoeiten)
- TGF\beta
- IL-10
- prostaglandin

**Gut macroophages**

Highly phagocytotic but do not produce ROS, NOS, inflammatory cytokines. Mostly because of *IL-10* that THEY produce constitutively. IL-10 also promotes survival/differentiation of returning effector T-cells.

### Antigens don't just pass through M-cells

- Between gaps in normal epithelial cells
- Antibody coated pathogens uptake by FcRn (neonatal Fc R)
- Transepithelial dendrites / arms between cracks from macrophages

### IgA

3-4g of IgA secreted a day. ~75K present in normal intenstine at a time.

- Class switching exclusively in organized lymph tissue by T_FH cells, mediated by TGF\beta
- Travel to lamina propria where differentiate into plasma cells.
- IgA travels into gut lumen by binding to pIgR (binds to J-chain characteristic of "polymeric" immunoglobulin molecules)

How does it work?

- Inhibits binding to epithelium
- Neutralizes bacteria in lumen
- Can do the same within lamina and reexport to the gut

IgA defficiency is common (1 in 500 individuals) but not terrible because IgM production ramps up / compensates.

### Weird Innate Cells

2/3% of lamina propria T-cells

MAIT

- recognize metabolites of vitamin B (riboflavin) presented on MR1 (MHC analogue with specialized binding groove for small molecules)
- limited repertoire of \beta chains

iNKT ?

### Intenstinal epithelium is unique compartment

Single largest population of lymphocytes in the body. 90% are T-cells and 80% are CD8.

- CCR9 + \alpha_E:\beta_7 (recall E-selectin)
- show restricted TCR clonality

There are two types:

a/ "inducible" - essentially normal cytotoxic T-cells

b/ "natural" - seem to kill epithelial cells and promote repair.

- express NKR that bind to MHC-like MICs that are expressed when epithelial cells are stressed
- can express \gamma-\delta or \alpha\beta but have a CD8 \alpha\alpha homodomier
- excess \gamma\delta IELs are associated with celiac disease
- unusual development. Likely escaped negative selection (autoreactivity) despite having autoreactive TCRs. Likely due to homodimer CD8 binding less tightly than the normal version.

## Response to infection

### Enteric pathogens cause local inflammation

Different receptors:

- TLRs on surface or with intercellular vesicles

- NOD
- NLR (NOD-like receptor)

Cause release of cytokines like IL-1/IL-6 and chemokines (eg. CCLC4-6) Specialized cells:

- Paneth cells release antimicrobial peptides (ILC3/T_H17 module)
- Goblet cells

**Active immune system kicks in**

Dendritic cells release cytokines and help effector T-cells develop

- in Peyer's patches, they migrate to T-cell area
- in lamina propria, migrate to mesenteric lymph nodes

B-cells (producing IgA) proliferate in Peyer patch + mesenteric lymph follicles

homing adressins?

**T-cells preserve epithelial integrity**

- CD8 kills epithelial cells to promote new cell growth from crypts
- IL-13 (ILC2) promotes turnover / shedding ("moving target")
- IL-22 (ILC3) promotes tight junctions

Peristaltic action (wave like muscle contraction of gut) that expels stuff

**Oral tolerance**

Migration of dendritic T-cells producing retinoil acid + TGF-\beta Expansion of Tregs that dampen immune response in the gut and systemically

eg. inability to generate Tregs or hyperactivity of T_H2 cells + IgE antibodies leads to peanut allergies

**Role of microbiome**

- Essential in breaking down molecules needed by enterocytes:
- Mostly SCFAs like butyrate
- Also cellulose, vitamin K
- Surgical procedures that remove this population lead to necrosis + inflammation of enterocytes due to lack of SCFA fuel
- Dysbiosis is the modulation of these populations

**Gut immune system control microbiota populations**

- Dendritic cells that ingest and respond to these bacteria are "not fully activated", preventing full deletion + maintaining some balance
- ~75% of gut microbes coated with IgA
- IL-10 produced by Tregs plays a role in preventing too much inflammation

**Gut microbes crucial for healthy immune system**

Not just gut, systemically. Why?

Specific mechanisms of immune modulation by bacteria:

- Flagellin (eg. lactobacilli):
  - TLR activation leads to IL-6/IL-23 -> T_H17 differentiation
  - IgA production
- Clostridium:

  - Butyrate leads to IL-10 production (dampens inflammation)

**IBD + Chron's**

- Combination of overactivity of immune cells + lack of suppressive factors
  - IL-23 is important - leading to T_H17 and sometimes T_H1
  - Linkage with polymorphism in IL-23 receptors
- Not linked to a specific species, but general pathology with bacterial communities

# Failures of Host Defense

# Failures of Host Defense Mechanisms

## Severe Combined Immunodeficiencies

Mutation in IL2RG gene on X chromosome (common gamma chain, \gamma_c) causes *SCID*:

- T-cells unable to develop
- B-cells are able to develop but are unable to function without T-cells

Variants:

- somewhat ablated RAG1/RAG2 lead to *Omenn's syndrome*. Limited T-cell repertoire somehow becomes very autoreactive leading to symptoms resembling graft vs. host disease. (called *hypermorphic mutation*)
- "radiation sensitive" SCID is when defects in DNA repair proteins (eg. Artemis) cause reduced somatic hypermutation that also increase susceptibility to radiation / general DNA damage

**SCID can be caused by pathologies in the purine salvage pathways**

- ADA (adenosine deaminase)
- PNP (purine nucleotide phosphorylase)

Purines - pyrimidine fused to imidazole Defficiency in purine salvage lead to accumulation of toxic purines and kill lymphocytes.

What does purine salvage do? Purine bases are used all over the place, not just in DNA:

- metabolism (cyclic AMP, ATP, cofactor for NADP, CoA)
- cell signaling (GTP used in G-protein signaling,
- protein synthesis (tRNA loading)

**T-cell based SCID (development)**

- "Nude" mice/people lack proper thymus epithelial formation (FOXN1).
- DiGeorge syndrome caused by large deletion of chrom 22 leading to similar thymic underdevelopment
- MHC-II deficiency is caused by at least 4 upstream regulatory genes
- MHC-I deficiency is generally caused by dysfunctional peptide transport proteins (TAP1/TAP2) but otherwise normal levels of mRNA + surface MHC-I. People with this are generally healthy indicating alternative ways of displaying peptide
- Dysfunction in thymal epithelial peptide presentation (AIRES) leads to autoimmunity

**B-cell based SCID (development)**

- Characterized by inability to fight bacteria with polysaccharide membrane components that need opsonization
- Ogden Bruton (1957) characterized X-linked absence of antibodies

- Bruton's Tyrosine Kinase (BTK) is essential in pre-BCR signaling. It is one of the downstream proteins when the heavy and nascent/decoy light chain assemble needed to show that it functions
- *XLA* X-linked agammaglobulinemia
- Women randomly deactivate one chromosome early in embryonic development, creating mosaic patchworks of cells. In *XLA*, only the B cells that receive the functional copy develop

Other components can be dysfunctional in rarer versions of XLA, in order or frequency: 1/ Igu 2/ lambda light chain 3/ Ig\alpha/Ig\beta (heterodimer with ITAMs necessary for internal signaling of BCR) 4/

**T-cell / B-cell activation based defficiencies**

Defects in class switching - *hyper-IgM syndrome*

On set grouped together by pathologies of CD40L - CD40 pathway:

- Most common is CD40L (on activated T-cells), necessary for eg. AID via CD40
- CD40L defficiencies also prevent crosstalk with macrophages / dendritic cells, dampening ILC1 response
- truly "combined" susceptability to pyogenic bacteria and intracellular bacteria

CD40 itself NEMO

Severe *Variable* ID are characterized by late onset, smaller issues usually with just a single isotype and scoped to B-cell:

- Mutations with TACI (interacts with APRIL and BAFF produced by T cells)
- Mutations with ICOS (present on activated T cells and necessary for B cell)
- Hyper IgE production (**Jobs syndrom**) - STAT3 dysfunction, leading to reduced ICL3 module. Lack of T_H17 cells might prevent inhibition of T_H2 and too much IgE

**Immune Module Cytokine Defficiencies**

- IL-12 + IFN-\gamma receptor mutation -> susceptibility to Salmonella, Mycobateria, Listeria
- IL-17F / IL-17RA mutation -> susceptibility to *Staphylococcus aureusa* and *Candida albicans*

**Malfunctions with cytolytic pathway**

Shows the complexity and number of moving pieces with effective release of eg. perforin:

1/ Acivation 2/ Polarization (movement of endosome to membrane) 3/ Docking 4/ Priming (making content of membrane "fusion competent") 5/ Fusion (of endosome with membrane)

Defects with intracellular trafficking:

- *Chediak-Higashi syndrome*
- *Griscelli syndrome*

Also cause issues with melanocytes (skin pigment) and vision (eye pigment) in many cases. Many

The lack of active immunity stimulates macrophages. Macrophages cause so much inflammation that they start eating blood cells

*hemophagocytic lymphohemocytosis*

**Failure to curb EBV**

- Epstein-Barr Virus infects 95% of people
- Usually reduced to a latent state within B-cells by healthy NK / T / NKT response
- *XLP* (*X-linked lymphoproliferative*) causes expansion of EPV infected B/T cells + lack of antibodies

T-cell <> antigen presenting cell NK-cell <> target cell *SLAM* mediates interaction dysfunctional SAP prevents SLAM

### Dendritic cells

T cells suffer

The two population validated genetic targets are:

### GATA2

Kind of unclear but probably involved in maintaining stem cell progenitors.

Has two forms:

- autosomal recessive (two bad copies)
- autosomal dominant negative (one bad copy)

### IRF8

Specific defect with dendritic cells rather than progenitor. DNA binding transcription factor.

### Complement

### Recall pathways

- Classic
- Mannose binding lectin
- Alternative (constant, low-rate "tick over" that provides blood surveillance)

All converge to C3 cleavage and membrane attack components C5-9.

### Different complement disease

*C3* - increased susceptibility to pyogenic bacteria

*Membrane attack proteins* (C5-C9) result almost exclusively in susceptibility to Neisseria

- Japan population - 1 / 2e6 -> 1/200 risk when these proteins are mutated

*classic complement* - more *immune complexes* (eg. clumps of matter in kidney? SLE)

*MBL* - (common, ~5% of population) more risk of bacterial infection

*control proteins* - complement covers self cells, eg. blood cells in paroxysmal nocturnal hemoglobinuria

### Phagocytic cells

These are generally bad. Four major types:

1/ Development 2/ Adhesion 3/ Activation 4/ Killing

### Development

- Severe Congenital Neutropenia (SCN)
- Cyclic Neutropenia

With SCN, 3-5 * 10^9 per liter to like 0.5 * 10^9

ELA2/*neutrophil elastase.*

- Mostly caused by defects in the "primary" granules (azurophilic granules, stained blue with Romanowsky/ methylene blue stains)

- autosomal dominant mutation causes one form of SCN
- Still don't undertand what causes the cycles (~21 day, 3 week periods?) in the cyclic flavor.

Variety of autosomal recessive variants

- Kostmann's disease

Can also gain neutropenia with therapies - eg. chemo, cancer, aplastic anemia (condition of bone marrow that cause defficiency in immune cell production) OR part of other combined ID.

**Adhesion** The migration to infection is a multi step process and any damaging any component can lead to problems

Rolling -> Firm adhesion -> activation and "lock" of integrin

LAD2 -> LAD1 -> LAD3

1/ glycosylated ligands needed for selectin mediated rolling 2/ missing piece of \beta integrin (CD18) 3/ induction of high affinity state of \beta integrin

**Activation** TLRs play central role. High amount of redundancy and remarkably only defects with TLR3 actually cause problems. Susceptibility to HSV and can cause brain swelling (encephalitis).

Also other PRR (pattern recognizing receptors)

**Killing / Ingestion** Defects in the ROS. Result in *chronic granulomatous disease*.

Actually showed that oxides themselve are insufficeint to kill but create a low pH environment that allow true microbicidal peptides to kill.

**Regulators of inflammation**

*Pyrin*:

- apoptosis of inflammatory cells
- inhibit secretion of IL-1\beta

*Inflammasome*:

Functions:

- releases inflammatory cytokines, by processing eg. pro IL-1\beta into active form
- causes programmed cell death, via cleaved caspase

Structure:

- NLRP3 is a "sensor" protein
- forms a pinwheel structure

Diseases:

- *Familial Mediterranean Fever* - lack of pyrin.
- *pyogenic arthritis, pyoderma gangrenosum and acne* (PAPA) - another lack of pyrin
- *TNF-receptor associated periodic syndrome* - lack of TNF receptor, high TNF-\alpha. Treated by TNF blockers, like those developed for RA.
- *Muckle-Wells / familial cold autoinflammatory syndrome* (FCAS) - defects in NLRP3, causing inflammasome to be "on" more than it should

### HSC transplantation

You can get autoimmunity in two broad directions, mostly caused by MHC mismatches:

- graft vs host: when immune cells from transplant attack host
- host vs graft: where immune cells in the graft attack transplant

Depletion by chemotherapy is common, but the extent or use of cytotoxic pre treatment depends on the disease. Some diseases require complete elimination of immune cells because they were already dysfunctional.

(like XLA, the B-cell abnormality that leads to HSV)

Somatic gene therapy began by the use of retroviruses to insert corrections. However, strong enhancers in genetic cargo misinserted upstream of proto-oncogenes, causing leukemia.

Broad corrections:

- ex-vivo editing, driven by iPSC reprogramming to make new HSC
- expression systems that can be shut down

## How different pathogens evade immune system

- HIV. virus
- tuberculosis (Mycobacterium). bacteria.
- malaria (Plasmodium falciparum). protozoa

### Extracellular evasion strategies

Gram negative. LPS. TLR-4. Gram positive. Peptidoglycan. TLR-2 + NOD1/NOD2.

Outside:

- Modifications to LPS lipid-A base/core. TLR-4 antagonism instead of agonism.
- Modulation of peptidoglycan.
- Carbohydrate shell to both evade TLR-2 and opsinisation/phagocytosis.
- Significant carb variation on eg. Streptococcus
- DNA rearrangement on E coli, Neiserria leading to carb / pillus variation

Misc.:

- Blocking C3 cleavage
- *Protein A*. Fc-binding proteins that interfere with antibodies
- *Factor H*. Prevent complement binding. Decorated on bacterial surfaces.

### Intracellular evasion strategies

- Prevention of phagolysosome formation
- Escape phagosome
- Resistance to microbicidal compounds
- Listeria escape into cytosol. Hijack actin push membrane bound packets to other cells.
- Salmonellaa release factors to prevent destruction of their vacuole
- Tuberculosis prevents fusion with phagosome

Two types of lepropsy, with different response types:

- Tuberculoid lepropsy
- Lepromatous lepropsy

**Protozoans**

Theme here is *evasion*

- Trypanosoma brucei constantly cycle the glycoprotein they express allowing variants to survive under selection of antibody. Cycles of antigen clearance eventually lead to coma ("sleeping sickness")
- Plasmodium moves from the liver to the blood cells, creating moving target for disease
- Leishmania major prevents release of IL-12 by macrophage host, suppressing NK activation + T_H1 differentiation in type-1 response

**RNA virus**

- plasmacytoid DCs + NK cells before T_H1 + CD8 T cells are ready
- RNA polymerases have higher error rate -> viral chromosome is smaller
- RNA chrom also tend to be segmented
- *antigenic drift* - mutation of epitope
- *antigenic shift* - exchange of segmented

Influenza - Hepatitis C virus (HCV), leading cause of liver cirrhosis

- CD81 binding glycosylated protein that is difficult to bind to

**DNA virus**

- Lower mutation rates -> larger genomes -> more machinery to evade/subvert
- Many enter latency allowing them to escape recognition for long periods with cyclic reactivation

9/10 people are infected by the 5 common types of herpesvirus:

- HSV-1 (mouth)
- HSV-2 (genitals)
- EBV (mononucleosis / "mono")
- varicella-zoster
- cytomegalovirus (CMV)

**Evasion Mechanisms** *immunoevasins* are class of proteins preventing peptide display

- Disrupting various steps of loading and presenting antigens (redirect to degradation in the ER) 1/ TAP transport/peptide entry to ER 2/ movement of MHC to cell surface 3/ *dislocation* (degradation of MHC)

Inhibiting cytolysis by NK cells normally caused by lack of MHC expression with decoy MHCs.

Cytokine disruption:

- Decoy receptors
- Receptor inhibitors
- JAK/STAT inhibition
- expression inhibition
- cyotkine induced TF inhibition
- direct antagonism of cytokine itself
- decoy cytokines, eg. cmvIL-10

Chemokine disruption:

- Decoy receptors (herpesvirus + poxvirus display >40 homologs)

CMV / HCV shown to increase PDL1.

Recall cheomkine receptors are exclusively GPCR (7 transmembrane spanning). While cytokine receptors are single transmembrane protein (also associated with STAT).

## AIDS

Zoonotic infection with at least four crossover events, two of which were in the early 1900s, likely from lowland gorillas or chimpanzees.

HIV-1/HIV-2

### Structure

*3 major genes*

*gag*

*pol* (alone encodes replication tools)

- reverse transcriptase
- integrase
- viral protease

*env* gp120 + gp41 (trimers alongside the envelope)

*6 regulatory genes* Tat + Rev are essential for early replication Nef Vif Vpr Vpu are necessary for efficient viral production

What determines if it enters latent or actively replicating state? Maybe determined by the state of the infected cell itself

CD4 cells are long lived in contrast to macro + DCs. Difference in state there provide clues for quiescence

### Replication mechanics

*NFkB + NFAT* both used to induce expression of proviral genes (nuclear factor of activated T cells) Recall:

- NFkB is common across most immune cell types
- NFAT is specific to T cells and primarily induced by direct TCR stimulation

(Bind directly to the promoters in the proviral LTR - these are functional!, not just scars for integrase recognition)

*Tat* and *Rev* reveal important eurkaryotic replication components:

- Tat phosphorylates RNA polymerase (in combination with cyclin T1 + CDK9), to enable the enzyme to produce full length transcripts
- Rev is necessary to shuttle unspliced mRNA for later viral components out of the nucleus. It binds to motifs on later unspliced transcripts and recruits

The smaller regulatory genes:

- decrease expression of surface proteins like MHC I/II, allowing invasion
- prevent cytidine deaminase (APOBEC) from mutating transcript

### Where in the body replication happens

Usually in sex fluid or blood. Free virus or within one of the cells listed.

*genital + gastrointenstainal mucosae* are primary sites of sexual transmission

- initial contact with memory T cells + DCs
- spread to lymph
- from the lymph the entire bloodstream

**Tropism**

Variants have affinity for *CCR5* or *CXCR5*. (a la R5 or X4).

Needs to get to the CD4 immune cells in the lamina propria. So *transcytosis* through epithelial cells + traveling through "interdigitating" DCs.

- gp120 binds to proteins on both squamous and columnar epithelia
  - squamous -> vagina mucous, penis foreskin, ectocervix, rectum, oropharynx, esophagous
  - columnar -> endocervix, rectum, upper GI
- DC receptors:
- langerin (CD207)
- mannose receptor (CD206)
- DC-SIGN

1/ acute phase (influenza like illness

- rapid replication of virus in mostly CCR5+ CD4+ T cells
- population quickly declines
- immune cell depletion in GALT leads to further infection (in particular IL-17
- IL-22 no longer around to promote tight epithelia) 2/ asymptomatic phase (~10 years)
- seroconversion (first point antibodies detected)
-

**Genetic resistance**

Non function variant of CCR5 in Caucasians confers some resistance (not cure but decreases rate of progress) and suggests some past selection. Some suggest small pox and bubonic plauge provided selection.

**Development of AIDS**

After the initial depletion, T cells recover. But after the variable period, they start to decline again:

- CD8 T cells kill virus infected T cells
- latent virus awakening + killing T cells
- disruption of new T cell development in thymus

Immune activity does help. Type 1 response (IFN-\gamma + granzyme B) inversely correlated with viral presence in blood ("viral load"). The irony is these are the very cells the virus kills off slowly...

Anti-retrovirals are somewhat effective

Neutralizing antibodies do develop but generally later (after first year). Vaccines therefore might be promising

ADCC mediated (non-neutralizing) antibodies also recruit NK, macrophage, neutrophils.

Virus mutates a lot, making antibody mediated response challenging

**Reservoir in lymph tissues**

- Within CD4 T cell, macrophage, neutrophil (latter are shorter lived and not major holders)
- Immune complexes actully form on the *surface* of follicular DCs in lymph

**Genetic resistance**

Some people (1 / 300) live with latent virus and never progress to AIDS. Strongest loci are HLA traits, presumably enhancing presentation of virus and preventing their silent latent state.

**What kinds of diseases**

- Candida (oral fungal infection)
- tuberculosis

Cancers:

- EBV induced lymphomas
- Kaposi sarcoma (induced by HIV)

In both cancers, the viruses don't actually introduce mutation but induce survival that select for oncogenetic mutation)

**How do we treat it?**

Target:

- reverse transcriptase
- integrase
- protease (required for creating functional product)

***HAART* (Highly active anti-retroviral therapy)** Can prevent infection of new cells. But cannot remove integrated provirus.

Combination therapy, eg. a cocktail of different inhibitors, called HAART. Showed success starting in 1995. Must take for life.

- Releases T cells trapped in lymph tissue
- Decreases CD4 cell killing by CD8 T cells
- Somehow new T cells emerge from thymus

Can detect these with TRECs (T cell receptor excision circles) - the little loops that form during somatic hypermutation.

New drugs also target viral integration + cDNA creation. Do not deplete latent reservoir

**Targeting reservoirs**

- Try to tease out virus by activating provirus. (Clincal data does not show efficacy over HAART alone)
- Cytokines (TNF-\alpha, IL-1) or histone acetylation to active proviral genes

Berlin patient HCST to inject T cells with CCR5 mutation. Progression free for 5 years. Risk of CXCR4-tropic variant mutation. Also impractical as population level cure.

**Vaccines are difficult** Two types of vaccines:

- Prophylactic. What variant to you target? If you do generate antibodies, it is hard for them to bind glycoproteins on HIV.
- Already infected. If you increase antibody + CD8 T cell response, still hard to clear virus.

Other vaccines use broken viruses of the type they are treating. Cannot do this with HIV because of high mutation rate + risk of recombination Use "canary" virus - AAV, vaccinia

delivery gag,pol,env genes with canarypox vector:

- only vaccine that has any efficacy
- data shows type of response useful - non-neutralizing for ADCC. (this is good because neutralizing kind are hard to develop)

# Allergy and Allergic Disease

# Allergy and Allergic Defense

Gell + Coombs classification system:

1. IgE (+ Mast cells)
2. Complement
3. Fc effector molecules
4. Various cellular effectors (lymphocytes + myeloid)

However, becoming more clear the 1/2/3 immune module system is a better classifier of allergies.

Burden of allergy disease is actually sig. Lost time from school + work.

## IgE Allergies

*Immediate hypersensitivity reactions* - multivalent IgE bridging on surface of mast + basophils.

Predisposition to IgE reaction is *atopy*

## Non-IgE Allergies

First exposure called sensitization.

- nasal passages (allergic rhinitis)
- eyes (*conjunctivitis*, refers to the conjunctiva)
- lower lungs + airways (asthma)
- gastrointestinal tract ()

Systemic reactions can spread from initial site:

- skin
- lungs (bronchospasms)
- vascular system

*atopic march* is the progression of reactions as someone ages

### Mechanisms of sensitization

1/ T_H2 2/ IgE class switching

1/ T_H2 differentiation

- DCs in mucosal tissue sample antigen and migrate to lymph tissue
- T_H2 cells induced by IL-4, IL-5, IL-9, IL-13
- T_H2 cells produce IL-9/IL-13, positive feedback
- Mast cells + epithelial cells produce IL-33, also helping differentiation

In healthy individuals + absence of inflammation, T_regs induced by , controlling the response.

2/ T_H2 cause class switching:

- IL-4 or IL-13 -> Jak1/Jak3 -> STAT6
- CD40L (T cell) <> CD40 (B cell)

Mast cells also present CD40L + secret IL-4.

Dendritic cells, like Langerhan cells in skin, can "trap" antigens with nets of surface IgE. eg. ($Fc\backslash epsilonR1$)

**Antigens that cause sensitization**

Actual numbers (small amounts):

- 1 ug of ragweed pollen allergen
- 20-75 ug bee venom

It is difficult to identify common characteristics of allergens.

Some are proteases: *Der p 1.* Allergy in 20% of people. Cleaves occludin in epithelial junction and access to subepithelial antigen presenting cells *LEKT1.* (lymphoepithelial Kazal type-related inhibitor). Allergy diseases caused by mutations in protease inhibitors. *SPINK5* in *Netherton's syndrome*

- overactive *kallikrein.* Shedding of keratinocytes from cleaved desmosomes.
- overexpression of TSLP, TNF, ICAM
- LEKT1 also inhibits Staph. See colonization of skin with these bacteria in Netherton's syndrome patients

Transfer of 2S albumin from brazil nut into soybean caused allergic reaction discontinuation of cell ag project

**Genetic predisposition**

*Atopic triad*:

- allergic rhinoconjuctivitis
- allergic asthma
- allergic eczema

Asthma + eczema often linked. Although each individual allergy type has own linked genes. Also ethnic differences.

**Risk factors for general atopy** *11q12-13*

\beta subunit of Fc\epsilonRI

*5q31-33*

1/ IgE class switching, mast + eosinophil activation

IL-4, IL-5, IL-9, IL-13

2/ TIM family

(T-cell, Immunoglobulin domain, Mucin domain) Associated with *airway hyperreacrtivity*, not only to specific allergens

Expressed by T_H1 + T_H2 cells https://www.nature.com/articles/nri1111

3/ p40

Common subunit of IL-12 + IL-23

4/ \beta-andrenergic receptor

Smooth muscle contraction in airways

**Risk factors for specific antigens** In contrast to above genes, which dysregulate immune response downstream of arbitrary antigen, these are MHC allele variants for specific allergens

eg. HLA-B15:02 + peptide react to carbamazepine (seizure medication) leading to rashes over the body that lead to scalding

**Specific allergy disease mutations**

- *fillagrin* (helps make "cornifying" keratinocytes tight) involved in eczema + asthma. 7-10% of Caucasians have fillagrin mutation.
- metalloproteinase *ADAM33* in bronchial smooth muscle cells

**Environmental risk factors**

Rural Africans show less atopy than American Africans.

*hygiene hypothesis* - exposure to pathogens early in childhood skews immune development towards type 1 immune response from type 2.

However, negative correlation between atopy + helminth infections. Hygeine hypothesis has evolved to include all three immune response types => trigger *TGF-\beta + IL-10* ->

- less differentiation of all three helper types and
- increased production T_reg

Some pathogens help and others increase risk:

- RSV skews IFN-\gamma towards IL-4 (1->2) and increase asthma development inkids

Diesel exhaust increases ROS, like ozone, increasing IgE production 20-50 fold

- dysfunction in genes that prevent oxidative stress (GSTP1, GSTM1) linked to worse asthma
- inhibitors for eg. NADPH, might help asthma

**T_regs play important role**

- T_regs isolated from atopic individuals when cocultured with T_H2 cells are less effective a repressing cytokines
- Mice defficient in Foxp3 have greater risk for atopy

Treatments:

- IDO
- IL-35 / Il-27 cyotkine therapy, which inhibit T_H2

## Mechanisms

1/ IgE bound to surface 2/ Mast cells release factors:

- histamine (from granules)
- prostaglandin
- leukotriene
- PAF (platelet activating factor) 3/ Physiological effect of factors 4/ Recruitment of effector cells

**Different IgE receptors**

*Immunoglobulin family Fc\epislonRI*

Mast cells + basophils Activated with familiar tyrosine kinase -> ITAM -> Syk -> downstream effectors Increased IgE increases surface expression

*C-type lectin family Fc\epsilonRII*

Many immune + epithelial cell types, including T + B cells.

**More on mast cells**

Mast cells => IgE inflammatory responses (Kit knockout mice do not produce these responses)

Activated by these factors: *stem-cell factor* / Kit ligand IL-3 IL-4 / IL-9 (type 2)

**Granule contents** Degranulation is an important component of function:

1/ histamine (short lived vasoactive amine) 2/ serine esterase (protease) -> tryptase, chymase, cathepsin G

- Histamine acts through 4 GPCRs.
- Histamine causes blood flow + vessel permeability.
- Leads to edema + local inflammation.

What do the proteases do? Activate metalloproteinase that destroy venom. Also break down tissue...

**Types** Location and protease content

*MC_T* -> tryptase, in mucosal epithelia (lamina propria) *MC_CT* -> tryptase + chymase, in submucosa (deeper than the lamina propria)

**Eicosanoids** Distinct from granule contents. These are lipid mediators synthesized from common pathway.

AA cleaved from membrane:

- *Cyclooxygenase* -> prostaglandin, thromboxane
- *Lipooxygenase* -> leukotriene

Prostaglandin is important in asthma C4,D4,E4 leukotrienes sustain inflammation

**Cytokines** TNF-\alpha - some stored and some synthesized Recruit immune cells (and cause epithelial cells to express adhesion molecules, helping recruitment)

IL-4 -> Type II

**Eosinophils**

- <6% of cells in healthy people
- IgG, IgE, CR1 + CR3 receptors
- Two main effector functions:
- granule contents - proteases and ROS
- synthesize mediators (prostaglandin, leukotriene + cytokines)

How do they cause allergies?

- Kill T_H1 cells (IDO)G. T_H2 expansion might be in part explained by this.
- Located in submucosa
- Few are present without infection

Production and migration are distinct.

- GM-CSF + IL-5 cause *production* in bone marrow
- *Migration* governed by *eotaxins*: CCL11, CCL24, CCL26

Mice can have eosinophilia systemically with just lots of IL-5.

CCR3 (eotaxin receptor) is quite promiscuous and can bind to other chemokines

Basophils:

- similar growth factors
- reciprocal control by cytokines

- eosinophil *major basic protein*

**Effects of IgE response**

Synthetic introduction of antigen (intradermal antigen challenge) in lab leads to two distinct stages:

*Immediate reaction* is characterized primarily by histamin acting on different tissues:

Airway

- vascular permeability + blood flow => edema
- edema + smooth muscle contraction => airway narrowing

Vascular

- extravasation (leakage of fluid from blood vessel) -> edema
- reflex vasodilation (increase of blood vessel size from drug). Acts on nerve endings, but effect is on vessels. *wheal-and-flare reaction* (wheal is swollen bump)

*Late-stage reaction* occurs 3-9 hours. Cellular recruitment and continued synthesis of factors

(Again primarily caused by synthetic high-dose antigen and unlikely to happen naturally)

*Chronic allergic inflammation* is the natural long timeline allergy state

Persistent T_H2 response.

T_H2 cells release *calcitonin* + *VEGF* with effect on blood vessels.

- Can actually change size and number of cells (hypertrophy + hyperplasia of *smoooth muscle* cells respectively
- *airway tissue remodeling*

Think of allergic response as starting from the site where mast cells degranulate.

**Anaphylaxis**

Mast cells are often associated with blood vessels Widespread release of eg. histamine on many vessels is *anaphylaxis*

Can also be caused by autoantibody, to IgE or Fc\epsilon,

Symptoms:

- *Utricaria* - distributed version of wheal + flare
- *Anaphylactic shock* - lowered blood pressure + constricted airways
- Can be treated with epinephrine or \beta-adrenergic receptor modulation, relaxing blood vessels

Penicillin and structurally related drugs can cause anaphylaxis.

Hapten - \beta-lactam ring (usually essential for antimicrobial activity) binds to host proteins to form covalent conjuages

- T cell response from self-protein conjugates
- B cell response (from penicillin conjuages activating BCR)
- Can cross link IgE on Mast cells

**Asthma + Rhinitis?**

- allergic rhinitis
- allergic conjuctivitis
- allergic asthma

All caused by mast cell degranulation in response to allergens (within) pollen. Edema Mucus (rich in recruited lymphocytes)

Characteristiscs of chronic inflammation:

- hypertrophy
- hyperplasia
- eventually fibrosis

Why is fibrosis a bigger deal than smooth muscle cell remodeling?

- more permanent
- does not respond to asthma medication
- progressive, positive feedback

There are different asthma endotypes (different cell types + molecules):

- common allergic asthma
- exercise induced asthma
- neutrophil predominant
- eosinophil predominant
- steroid resistant sever

How do you get which type? Allergen sensitized with + genetic factors

**Mechanisms of common asthma**

- Familiar mast thing

Epithelial cell stimulation directly by TLRs:

- *IL-25, IL-33* (This explains where some of the type 2 cytokines even come from)
- CCL5 / CCL11 <> CCR3 on T_H2, eosinophil, basophil
- ILCs activated and express the familiar array (4, 5, 9 , 13)

Important facts about this biology:

- TGF-\beta helps drive airway remodeling of epithelial cells
- IL-9/IL-13 leads to goblet cell metaplasia of epithelial cells (replacable differentiation into mature cell type)
- iNKT cells (CD1d-restricted invariant) somehow are involved

You can actually get allergic disease without allergen. Shown with mice defficient in T_H2.

Similarly chronic inflammation triggered by allergen can continue by other factors:

- smoke, sulfur dioxide
- virus, bacterial infections of respiratory tract

Rhinovirus infection is main source of asthma hospitilizations / death

**Food allergies**

- IgE mediated
- non-IgE mediated (celiac)
- intolerance (metabolic)
- idiosyncratic (unknown)

Peanut allergies Mast cell in gut -> fluid movement + muscle contraction Systemic effects lead to urticaria, asthma, systemic anaphylaxis

Because allergens are generally pepsin resistant, adults who suppress this with (eg. antacids) may have late onset allergies

## Non IgE Mediated Allergies

Type 1 and Type 3

These aren't really allergic diseases?

### Drugs covalently bind to cells

Platelets - *thrombocytopenia* Red blood cells - *hemolytic anemia*

Cleared by Fc\gamma receptors from macrophages in the spleen.

### Immediate antibody mediated hypersensitivity

Conserved steps:

1/ Antibodies clump with antigen 2/ Complement (*C5a* + *C3a*) are activated 3/ Leukocytes w/ *Fc\gammaRIII* + CR activated; widespread tissue damage.

Soluble antigens can lead to large clumps of immune complexes:

- large clumps are cleared by monocytes (by fixing complement)
- the smaller ones get deposited on blood vessel and cause damage

*Arthus reaction*

Local immune response to spot in skin where antigen injected.

*Serum sickness*

- Originated when immunized horse serum was used to treat disease like *Streptococcus pneumoniae*
- 7-10 days after injection for class switched IgG reaction to occur (Normal class switching takes 3-5 days. Antigen processing + multiple discrete antigen recognition is involved in lengthening the timeline.)
- Same steps as above, leading to mast cell degranulation

**Serum sickness in monoclonal tx** Can result from improperly humanized monoclonals (eg. in anti-TNF-\alpha HUMIRA for RA).

Humanization fails for patients that have strange allotypes (constant region variations). One effect is more rapid clearance of tx and reduced effect.

- Bacterial reaction persist and continue to produce antigen
- Air pathogen exposure from dust/mold -> *farmer's lung*

### Delayed-type cell mediated hypersensitivity

T_H1 + CD8 T cells

*Mantoux test* - test for tuberculosis. Inject small amounts of tuberculin. Elicits local type I response.

***allergic contact dermatitis*** Like IgE, there are two phases: 1/ sensitization and 2/ elicitation

Langerhan cells, specialized APC / DCs, mediate sensitization in cutaneous allergies.

T_H1 response cyotkines in detail:

- IFN-\gamma: Macrophage activation + adhesion molecule expression on blood vessels
- TNF-\alpha + lymphotoxin: Local tissue destruction. Also adhesion on vessels.
- Il-3/GM-CSF: monocyte production in bone marrow

(*haptens* - small proteins that are immunogenic after being bound to carrier proteins. Eg. penicillin or )

Poison oak causes CD8 mediated skin dermatitis with soluble proteins that pass through membrane + present on MHCI. Comes from *urushiol oil*.

Insect bites can cause delayed-type hypersensitivity - itchy bump. (Also cause urticaria, swelling, anaphylactic shock from immediate hypersensitivity)

Metal ions (divalent cations)

**Examples of n-valent cations** Univalent cations: Have a charge of 1, such as the Cs+ cation Divalent cations: Have a charge of 2, such as the Ca2+ cation Trivalent cations: Have a charge of 3, such as the Fe3+ cation

#### Celiac disease

Both allergic and autoimmune disease.

Pathology:

- Loss of finger-like villi formed by epithelial cells
- Hyperplasia of crypt cells at elbows of epithelial folds

\alpha-gliadin -> deamidation by transglutaminase (tTG) -> binds to HLA-DQ2

Autoantibodies found against tTG but don't seem to do anything.

Why don't we get oral tolerance, eg. T_regs. Not all HLA-DQ2 variant patients develop celiac disease so not that molecule alone.

- other unknown genetic factors (down syndrome 6x more likely)
- gliadin peptides might cause epithelial cells to release IL-15 + express MIC-A -> NKG2D mediated CD8 T cell -> intenstinal damage + more causal CD4 T cell activation

# Autoimmunity and Transplantation

# Autoimmunity and Transplantation

Allergies are hyper immune response to foreign antigen.

Here we focus on immune reactions to self, bacteria and transplants.

## Mechanics of self-tolerance

We try to screen out self-reactive lymphocytes during development but:

- some weak interacting ones also respond to foreign antigens, so we need them
- some just escape

#### Review of tolerance

Paul Ehrlich discovered / described autoimmunity in early 20th century. Named "horror autotoxicus".

Autoimmune conditions are actually on the rise + comprise ~5% of population.

How does immune system identify "self" in the first place?

Central tolerance:

- broadly inactivation due to strong binding signals in thymus

Peripheral tolerance

- Antigens presented from normal cell turnover
- Not associated with cytokines or costim expression
- Either the activated T cell does nothing or T_regs induced

**More complete list of tolerance mechansims**

- *Anergy* (peripheral inactivation by signaling without inflammation or costims)
- *Central tolerance* (deletion by negative selection)
- *T_regs* (iT_reg/nT_reg, suppression in antigen or nonspecific way)
- *functional deviation* direct differentiation into T_reg

We have some amount of autoreactive lymphocytes in the first place. Why?

- positive selection requires loose binding to understand if MHC works
- constant stimulation in periphery from loose interaction to keep cells alive

**Central tolerance**

Thymus epithelial cells and CD8\alpha+ DCs express antigen genes from elsewhere in the body (like insulin).

AIRE (autoimmune regulator) TF seems to cause this.

*APECED* -> disease of pancreas autoimmunity from deffective (Autoimmune Polyendocrinopathy-candidiasis-ectodermal dystroophy)

- *interaction with TLR* - unmethylated CpG can be internalized by B cells and interact with internal TLRs
- autoantibodies against DNA, chromatin + ribo
- *antigen amount* - release of cardiac antigen following heart attack leads to immune response days later
- *antigen structure* - IgG can crosslink B cells when it forms immune complexes

**Priveleged sites**

Brain, eye, testis, uterus

How do these sites reduce immune activity?

- tissue barriers prevent lymphocytes from entering
- TGF-\beta induces T_reg differentiation
- Fas ligand

Sites source of autoimmune disease (MS): T cells activated elsewhere. *Sympathetic Ophthalmia*: trauma to one eye creates antigens that active T cells outside and allow them to come in (they are able to get through barrier somehow)

**Autoimmune disease controlled by specific T cell types**

different diseases depend on different T cell types *type 1 diabetes mellitus* - T_H1 *psoriasis* - T_H17

immune modulation

**Involvement of T_regs**

nT_reg - express FoxP3. Respond to self antigens specifically. iT_reg - develop in periphery in response to TGF-\beta. Not antigen specific.

Oral tolerance to antigen primarily caused by iT_reg generation

- Normal levels of defective T_reg present in MS patients
- Foxp3 negative (but IL-10 positive)

## Autoimmune disease

Often involve multiple components of system.

**Specific antigens cause disease**

Antigen + adjuvant injected into animal elicits disease. But models are not faithful because we generally don't know how autoimmunity starts

Organ specific:

*Hashimoto's thyroiditis Grave's disease*

Systemic:

*SLE Sjogren's syndrome*

Commensal:

*IBD* - Chron's + UC

**Involve different components of immune system**

- autoantibodies block receptors
- immune complexes deposit + cause tissue damage
- effector T cells cause inflammation + tissue damage

Antibody mediated disease can be transferred to newborns across placenta. Some can cause tissue damange before successful clearance of blood plasma with *plasmapheresis*.

**Autoimmune disease with strong antibody component** Myasthenia gravis - acetylcholine receptor Graves' - TSH (thyroid stimulating hormone) receptor Thrombocytopenic purpura - platelets

**Autoimmune disease mix all components of immune system** Examples. T cell mediated disease, like SLE + MS involve B cells (present antigen to T cell) and antibodies (generally around).

Antibody mediated disease involve T/B cell through antibody development (secretion + cross linking).

**How do you get chronic disease?**

Initial response to antigen straightforward enough.

Removal of the antigen in normal immunity usually causes effector cells to die off. In autoimmunity, either because of quantity or ubiquity, antigen always around.

How does the immune response amplify?:

- Big component is the breakdown of "sequestration" by intial tissue damage.
- More epitopes on same antigen are recognized (*epitope spreading*):
  - Hidden components (present in low concentrations) are efficiently presented by B cells
  - Additional molecules recruited with original epitope are internalized accidentally by B cells

Examples of epitope spreading:

- *SLE* - B cell internalizes large DNA complex. Activates T cells for differnt pieces - histone pieces, ribosomal protein
- *Pemphigus vulgaris* - binds to desmosomes and causes dissolution of skin tissue. Starts with harmless Dsg-3 antibodies but spreading eventually creates the kind causing deep skin blistering.

**Autoimmune disease organized by response type**

Like allergies, we first thought grouped by degree of active/inactive Becoming clear that similar to allergy re-classification - most of the immune components are involved in each response.

However we still classify based on *antigen* and *main mechanism*.

Classified based on antigen and main mechanism

- Type 2 responses play almost no role in autoimmunity, exclusively atopy/allergy

**Autoantibodies kill blood cells**

- Auto IgG and IgM bind to Fc and CR
- Rapidly cleared by *mononuclear-macrophage phagocytic system* in spleen
- or lysed by membrane attack complex of complement
- Autoantibodies against surface proteins (eg. autoimmune thrombocytopenic purpura)
- Nucleated blood cell lysis is less common but still happens

Tx strategies:

- *Removal of the spleen* to prevent macrophage clearance is a common tx strategy
- Also introduction of lots of nonspecific IgG (IVIG) - intravenous immunoglobulin.

**Fixation of complement**

- Can directly lyse cells
- Sublytic doses also causes problems:
- cytokine release
- respiratory burst
- arachidonic acid cleavage

*C5a itself is a chemokine*

**Nucleated cells are more resistant to complement lysis** Why?

In general, unnucleated cells lack metabolic machinery (mitochondria + nucleus) to make new proteins.

1/ Complement regulating surface proteins CD55 - DAF (decay-accelerating factor), accelerates C3 convertase decay CD59 - protectin (), binds c8/c9 components of membrane attack complex (MAC)

2/ Can repair some membrane damange caused by MAC

**Autoantibodies block receptors**

Examples of either:

Agonist (*Grave's disease*). Thyroid-stimulating hormone (TSH) from pituitary is controlled by thyroid hormone. Autoantibodies directly stimulate thyroid receptor and prevent negative feedback.

Antagonist (*myasthenia gravis*). Muscle contraction inhibited by autoantibody inhibition of nicotinic acetylcholine.

**Autoantibodies cause damage in ECM**

- Goodpasture's syndrome - type IV (basement membrane) collagen in renal glomeruli and pulmonary alveoli

Immune complexes:

- serum sickness (overwhlemed by immune complex)
- bacterial endocarditis (lodged in cardiac valve, unable to kill source)
- *mixed essential cryoglobulinemia*
- SLE

SLE main steps:

1/ Autoantibodies against proteins in nucleated cells ( 3 types:

- nucleosome subunits of chromatin
- spliceosome
- ribonucleoprotein complex ) 2/ Complexes traffic to renal glomerular basement membrane 3/ Poor clearance (due to dysfunction of complement *proteins* and lack of opsonization)

*Cryoglobulins*, soluble antibodies because of hydrophobic regions or clumping, that precipitate in joints/tissue.

#### T cells in autoimmunity

Hard to study because adoptive transfer in people require MHC matching

Culture requires the exact tissue from patient presenting problematic antigen

**MS** Multiple sclerosis is a response against nervous system myelin angitens:

- myelin basic protein MBP
- proteolipid protein PLP
- myelin oligodendrocyte glycoprotein MOG

These live on oligodendrocytes (support glial cell that maintain myelin sheath).

1/ Initial antigen stimulus 2/ BBB is "opened up". Epithelial cells lose tight junctures in response to inflammation and express adhesion molecules 3/ Rolling and entering (diapedesis) 4/ Re-encounter antigen

The plaques come from combination of demylenation and astrocyte proliferation / ECM production.

## Genetic and Env Basis of Autoimmunity

#### Certainly genetic component

For whatever reason, female occurence higher

GWAS identifies causal variants

#### Classifying causaul variants

- autoantigen availability or clearance
- apoptosis (regulate immune response) (FAS)
- signaling thresholds for T cell activation (PD-1 / CTLA-4).
- cytokines
- costims
- T_regs (Foxp3 mutation)

#### Monogenic autoimmune disorders

T_reg:

- Foxp3
- CD25

Monogenic Fas mutations

#### Role of MHC in diabetes

HLA-DRX alleles linked to type 1 diabetes

Genetic linkage of DR3/DR4 to DQ\beta (the actual gene with offending polymorphism)

Actually great case study for the loose binding hypothesis for T cell development

TODO: revisit dual nature of autoreactive display

Two hypothesis for HLA mutation and diabetes

1/ Inability to induce self tolerance during negative selection (from DC presentation) 2/ Or inability to create slightly autoreactive T cells that pass positive selection (these slightly autoreactive T cells are then assumed to have a regulatory function?)

**Chron's Disease**

Think about layers of innate immunitity between commensal bacteria and inner layers of epithelia:

- mucus from goblets
- epithelial junction
- antimicrobial peptides from *Paneth cells*
- phagocytic macrophages
- T_regs

With CD, you see dysfunction in one or more layers. Indirectly, this stimulates chronic T_H inflammation.

1/ NOD2 is an intracellular receptor that triggers inflammation (and release of antimicrobial granules in Paneth) 2/ CXCL8 dysfunction + lack of neutrophil accumulation 3/ Autophagy genes, ATG16L1 / IRGM, prevent clearance of swallowed microbes

Can also get upregulation of T_H response directly (eg. mutation in IL-23 for T_H17)

**Non genetic covariates**

- Vitamin D levels (higher incidence of autoimmunity in Northern Hemisphere). Suppress T_H17 levels.
- 'hygiene hypothesis' - exposure to microbes

**Mechanism of infection inducing autoimmune reaction**

General activation of lymphocytes from different (pathogenic) antigens Tissue damage released stored self antigens

**Molecular mimicry can induce autoimmune disease from infection**

- Mice can develop diabetes after viral infection

Why are the self-reactive lymphocytes there?

- Some escape deletion as described previously
- Antigen present in much greater quantity activates these normally ignorant cells
- *Autoimmune hemolytic anemia* follows *Mycoplasma blood infection*
- *Rheumatic fever*. Similar epitopes of *Streptococcus pyogenes* to self leads to antibody + T cell mediated tissue damage, including heart valves + kidney
- *Lyme disease*. Borrelia burgdorferi. Infection followed by Lyme arthritis.

**Drugs**

- Procainamide, for heart arrhythmias, induces autoantibodies
- Several lead to *autoimmune hemolytic anemia*
- Heavy metals, gold + mercury (mostly through haptenization)

**Stochastic cause**

Precise timing of infection with chance lymphocyte clones might be all needed. The pathogen / antigen might be different but lead to same disease.

## Alloantigens + Transplant Rejection

- MHC mismatches almost always trigger a response
- immunosuppression + transplantation medication helps
- blood is less problematic because express small amounts of MHC I (and no II)
- To avoid, antibody destruction, patients must be matched for ABO and Rh group antigens

### Graft rejection is mediated by T cells

Basic terminology:

- autograft - from same organism
- syngeneic graft - from genetically identical organism
- allograft - from different organism
- *acute rejection* occurs *10-13* days after transplantation. (in contrast to hyperacute or chronic).
- T cell role clear in lack of response in nude mice. Acute rejection can be stimulated in nude mice by adoptive transfer.
- *accelerated rejection* occurs 6-8 days second time from clonal expansion of primed memory T cells

### Rejection mostly caused by MHC mismatch

Actually origin of molecule name. Significant portion of T cell repertoire are reactive against nonself MHC

Immunosuppression makes MHC matching irrelevant for non bone marrow allografts (?).

Even HLA matched relatives experience rejection from non-MHC proteins.

Success in clinical transplants result of immunosuppression.

### Minor histocompatibility antigens

The set of proteins that cause graft reactions that are not MHC are called *minor histocompatibility antigens*.

*H-Y* responses come from proteins on Y chromosome (eg. *Smcy*). Male anti-female response (but not vice versa)

Most are autosomal and have yet to be characterized.

### Two ways of alloantigen presentation

1/ Donor origin APCs migrate to lymph tissue. *Direct allorecognition* Interestingly, migrating cells move through blood not lymphatics because solid organ allografts disrupt functioning lymph system.

2/ Uptake into recipient's own APCs. *Indirect allorecognition*. Key here is self MHC molecules do the presenting.

### Hyperacute graft rejection from antibodies

Complement dependent reaction occurs in minutes

- *ABO-matching*
- *cross matching* - looking for reactivity against donor white blood cells

Antibodies occlude vessels connecting organ graft to rest of body, leading to purple color and enlarged state.

Some transplanted organs less susceptible to ABO incompat + cross-matching

- *alpha-Gal*, surface carbohydrate
- hyperacute rejection occurs quickly with xenografts

- exacerbated because complement regulatory proteins (DAF, CD59, etc.) work less efficiently across species boundaries

**Why do allografts have an expiration date?**

*chronic allograft vasculopathy*:

- concentric arteriosclerosis
- hypoperfusion (cut off nutrients, blood)

Caused by recurring rejection events by antibodies + T cells

Some specific examples of tissue destruction:

- 'vanishing bile duct syndrome' in liver
- 'bronciolitis obliterans', accumulation of scar tissue in bronchioles

Other examples of failure causes:

- ischemia-reperfusion injury. Sudden blood flow can lead to tissue damage
- viral infections from immmunosuppression
- same disease in allograft as original organ

**Organ transplants are now routine**

Kidney first transplanted organ in 1950s (between twins).

- Kidney (17.8K)
- Liver (6.7K)
- Heart (2.7K)

Cornea (45K HSC (20K)

Corneal transplants are particularly successful because of lack of vasculature: harder for lymphocytes to move around and react to antigens.

Organ availability Progression of same disease Immunosuppression can lead to infection or cancer

1/ More targeted immunosuppression that does not lead to cancer 2/ Graft tolerance 3/ Xenografts

**Some autoimmune drugs** *Cell surface antibodies*

- Rabbit anti-thymocyte globulin (rATG). Polyclonal molcules made in rabbits against human T cells.
- anti-CD52 (*alemtuzumab*). Mature lymphocytes (eg. T / B / NK) marker for depletion.
- anti-CD3 (*muromonab*). Binds to TCR-CD3 complex for depletion.
- anti-CD25 (*basiliximab*). IL-2 mediated proliferation (proliferation/survival pathway).
- CTLA-4-Ig Fusion (*belatacept*). CTLA-4 binds B7, competing with CD28 on T cells. Ig region is for stability of long half-life in blood.

*Calcineurin inhibitors*

- Cyclosporine (CsA)
- tacrolimus

*Inhibit phosphatase required for nuclear translocation of NFAT.* Recall calcium dependent serine phosphotase. TCR increases calcium concentrations in the cytoplasm.

*mTOR inhibitors*

- Sirolimus (Rapamycin)
- Downstream of IL-2/CD25 signaling
- CD25 -> mTOR -> cyclin/CDK

*Antiproliferative drugs*

- azathioprine. *Inhibits purine synthesis, blocking replication*
- mycophenolate. *Inosine monophosphate dehyrogenase*

*Corticosteroids*

- Prednisone

Think of major function as downregulating eg. IL-2, for proliferation

#### GVHD

- Remove host T cells with chemo / radiation
- Introduce marrow graft
- Mature lymphocytes in donor marrow attack

Can be demonstrated with *MLR*

In leukemia patients, GVH is actually good for clearing cancerous lymphocytes *graft-versus-leukemia* effect

One strategy is *donor* T cell depletion. But can cause immunodefficiency + opportunistic infection if overboard. Also might be bad for GVHD.

Rather than depleting T cells, deplete the APCs. Still functional immunity. Graft-vs-leukemia more unclear.

#### T_regs

Supplementing CD25+ T_reg reduces symptoms or even prevents death from GVHD

(Though conventional T cells will express CD25 when activated, persistent high CD25 expression is a hallmark of T_reg.)

(Anergic T cells lack CD28 expression)

#### Why does the fetus not get rejected

- physical separation of T cells from mother by *trophoblast* (placenta outer layer)
- No MHC II + Limited MHC I on trophoblast
- Nutrient depletion (IDO)
- Regulatory cytokines - TGF-\beta + IL-10. (iT_res might have evolved from maternal tolerance) in environment
- Stromal cells of decidua

# Manipulation of the Immune Response

# Manipulation of the Immune Response

Small pox Several classes of immune modulatory drugs

## Immune Drugs

The main goal of these drugs is to prevent unwanted tissue damage, towards maintaining tissue function.

Conventional immunosuppresive drugs:

- corticosteroids (prednisone)
- cytotoxic (azathioprine)
- noncytotoxic but signal modulators (cyclosporin, rapamycin)
- cytokine receptor modulator

Precision proteins:

- mAbs
- fusion proteins (CTLA-4-Fc)

### Corticosteroids

Part of glucocorticoid family (`{mineralcorticoids, sex_steroids, glucocorticoid}`)

*prednisone* - a synthetic version of cortisol.

Activated intracellular receptors move into the nucleus where they control ~20% of genes leukocytes express.

**Main mechanism:**

Expression of AnxaI - inhibitor of phosophlipase A2 (Arachidonic Acid). Suppresses prostaglandin + leukotrienes.

Adverse effects:

- fluid retention (weak mineralocorticoid effects by upregulating sodium transporters in kidney)
- weight gain (promote fat creation and stimulate appetite)
- diabetes (increase glucose generation in liver + decrease insulin sensitivity in peripheral tissue)
- bone mineral loss (*osteoporosis* - bone cell proliferation, reduce calcium absorption)
- thinning of skin (reduce collagen + ECM protein production)

### Cytotoxic drugs

These guys mostly interfere with DNA synthesis and originally created to treat cancer. But found to suppress lymphocytes too.

High doses are only used to completely deplete lymphocyte populations eg. in HSC transplants.

*azathioprine*:

- converted to purine analog 6-thioguanine (6-TG) and competes with purine metabolism precursor to prevent A + G synthesis
- 6-TG can also directly incorporate itself into DNA where it increases risk of mutation when exposed to UV
- Competes with GTP for GTPase Rac1 and inhibits CD28 costim. Inhibiting this anti-apoptotic pathway leads to cell death.

*mycophenolate mofetil*:

- metabolized to mycophenolic acid: inhibits inosine monophosphate dehydrogenase blocking GMP (guanine monophosphate)

*cyclophosphamide*:

- becomes *phosphoramide mustard* and alkylates DNA with two "arms"
- part of nitrogen mustard family derived from mustard gas
- causes bladder hemorrhaging + inflammation

**What is a mustard?** alkylating agents. Usually central nitrogen or sulfur with two alkylating "arms".

Side effects come from general tissue toxicity.

### Cyclosporin A, tacrolimus, rapamycin + JAK inhibitors interfere with signaling pathways

### Calcineurin inhibitors

- cyclosporin A - Tolypocladium inflatum. Norway soil fungus. Binds to *cyclophilins*.
- tacrolimus - Streptomyces tsukabaensis. Japan filamentous bacteria. binds to *FK binding proteins*

Both bind to class of intracellular proteins called *immunophilins*. These are isomerases, but unrelated to mechanism. Rather form a complex and inhibit calcineurins.

Ca+ -> calmodulin -> calcneurin -> NFAT

Immunosuppresants of choice in clinic

**mTOR inhibitor**

- rapamycin - Streptomyces hygroscopicus. 'Rapa Nui' (polynesian name for Easter Island). Origin of name.

Downstream of PI 3-kinase + Ras/MAPK

mTOR1 (rapamycin specific)

- proliferation
- autophagy (clears out damaged cell components, like mitochondria)

mTOR2

- actin cytoskeleton remodeling
- adhesion
- migration

Rapamycin:FKBP disrupts mTOR1

**S1PR** Recognition of S1P by S1PR (actually a GCPR) *Fingolimod* (2010) is a competitive binder

**Jakinibs** Four members of JAK family: JAK1/2/3 + TYK2.

- Tofacitinib -> JAK3
- Ruxolitinib -> JAK1/2

Non mAB:

- late 1800s equine sera for diptheria and tetanus
- *anti-lymphocyte globulin* (pooled from exposure to human lymphocytes in mice)
- *IVIG* (pooled from many human donors to provide wide repertoire)

The use as targeted tx agents for specific immune components is recent development: *1986 - muromomab (OKT3)*

Anti-CD52 mAB *alemtuzumab* now used. Still use anti-lymphocyte globulin - sometimes broader depletion favorable, cost and clinical familiarity

Broadly *depleting* and *non-depleting*.

**Humanization**

Stem from the use of animal models to generate the antibodies

- *chimeric antibodies*: splicing human constant region into mouse variable region
- generating antibodies for genetically engineered mice with human genes
- fully human monoclonals from human hybridomas

**Why is maintaing culture of antibody generating B cells difficult**

- plasma cells (antibody generating B cells) isolated from donors are terminally differentiated and short lived
- to induce long life, viral insertion of oncogenes OR fusion with cancer cells directly is needed:
  - EBV transformation
  - hybridoma (fusion with myeloid)

omab - fully murine ximab - *chimeric*: CDR + framework fused with human constant zumab - *humanized*: only CDR spliced with human constant + framework umab - fully human

**Antibody table**

Rituximab CD20 "Eliminates B cells" "Non-Hodgkin's lymphoma" Alemtuzumab CD52 "Eliminates lymphocyte" "Chronic myeloid leukemia" Muromomab CD3 "Inhibits T-cell activation" "Kidney transplantation" Daclizumab IL-2R "Reduces T-cell activation" "Kidney transplantation" Basiliximab IL-2R "Reduces T-cell activation" "Kidney transplantation" Infliximab TNF-\alpha "Inhibit inflammation" "Chron's" Certolizumab TNF-\alpha "Inhibit inflammation" "RA" Adalimumab TNF-\alpha "Inhibit inflammation" "RA" Golimumab TNF-\alpha "Inhibit inflammation" "RA" Tocilizumab IL-6R "Block inflammation from IL-6" "RA" Canakinumab IL-1\beta "Block inflammation from IL-1" "Muckle Wells syndrome" Denosumab RANK-L "Inhibits activation of osteoclasts by RANK-L" "Bone loss" Ustekinumab IL-12/23 "Inhibits inflammation caused by IL-12 + IL-23" "Psoriasis" Efalizumab CD11a "Block lymphocyte trafficking" "Psoriasis(Withdrawn)" Natalizumab "\alpha_4 Integrin" ""Block lymphocyte trafficking" "MS" Omalizumab "IgE" "Removes IgE" "Chronic asthma" Belimumab "BLyS" "Reduces B-cell responses" "SLE" Ipilimumab "CTLA-4" "Increases CD4 T cell response" "Metastatic melanoma" Raxibacumab "Bacillus anthracis protective antigen" "Prevents actions of anthrax toxin" "Anthrax infection"

**Antibodies for allograft rejection**

- *anti-CD52 Alemtuzumab* depletes T cells but still maintains some graft-vs-leukemia effect (for unknown reasons)
- *anti-CD3*: *muromomab* no longer used because of cytokine storm from reaction with constant chain. *teplizumab* has a two alanine substitution preventing this.
- *anti-CD25*: subunit of IL-2. *daclizumab* + *basiliximab*.
- *CTLA-4-Ig fusion*: *abatacept* prevents costim (also approved for RA)

**Antibodies for autoimmune**

**Cytokines** TNF-\alpha:

mAB

- *infliximab*
- *adalimumab*

*etanercept*: TNF receptor subunit-Fc fusion

CD, ankylosing spondylitis, psoriatic arthropathy

IL-1

*ankira*: recombinant protein blocking IL-1 receptor Muckle-Wells, RA

IL-6 *tocilizumab*

INF-\beta Straight up cytokine: *Avonex* How does it work?

- inhibits components of inflammasome (cleave Il-1 pro-protein)
- reduces expression of IL-1 pro-protein

**Migration** integrin \alpha_4:\beta_1 (VLA-4) (binds to VCAM-1)

integrin \alpha_4:\beta_7 (binds to MAdCAM-1)

*natalizumab* specific for \alpha_4, inhibits both CD, MS

But causes infection, including brain JC virus

**Costims** CD28 (and B7 or CTLA-4) *abatacept* - CTLA-4-Ig RA, psoriasis

CD2 (CD58 or Lymphocyte Functioning Antigen, LFA-3) *alefacept* - CD58-IgG1

**Statins**

- Inhibits HMG-CoA reductase, reducing liver cholesterol biosynthesis -Somehow increases MHC-II expression on lymphocytes, likely due to lipid concentration changes in cell membrane

**Vitamin D_3** Bind to Vitamin D Receptors, which become TFs for a range of cytokines: IL-12 (DC), IL-2 + IFN-\gamma (CD4).

The doses needed cause hypercalcemia and bone resorption. Investaging alternatives.

**Using antigens**

- Small amounts of allergens shift response from IgE to IgG/IgA
- Oral ingestion of antigens in mice has shifted CD4 autoimmmune response to T_regs
- Limited success in people

Rather than oral ingestion:

- *Copaxone* contains 4 amino acids in ratios that mimic MBP (myelin basic protein). It induces T_H2 response + reduces relapse by 30%
- "altered peptide ligands"

## Cancer

**IO developed after mouse models**

*Congenic mice*: Identical except for single genetic region Historically, MHC-congenic strains developed after induction of tumor.

*Syngeneic tumor* (genetically identical donor) always grow differently. Introduction of irradiated tumor cells confer some immune response, specific to tumor. *Tumor rejection antigens*

**Mechanisms of tumor escape**

- Paul Ehrlich, 1908 Noble Prize, first to suggest immune system kills cancer
- Frank Burnet + Lewis Thomas, 1960 NP, formulated 'immune surveillance':

Elimination -> Equilibrium -> Escape

Evidence:

- Mice: lacking perforin (lymphomas), lacking RAG + STAT1 (gut epithelial + breast), lacking gamma/delta (skin cancer)
- Transplant: melonoma develop 1/2 years after kidney transplant, *post-transplant lymphoproliferative disorder* from unsuppressed EBV expansion

1/ Loss of antigen: mutations prevent expression of antigens in the first place 2/ Treated as self-antigen: antigens presented with lack of co-stims tolerize T cells 3/ Low immunogenicity: lose MHC expression, adhesion molecules, co0stims 4/ Immunosuppresive molecules: TGF-\beta (discovered in this context, hence name), IL-10 (suppressive, eg. DC in gut), IDO (degrades tryptophan), PD-L1 5/ Physical barrier

*MDSC*s (Myeloid Derived Suppressor Cells): pathologically activated immature myeloid cells. Suppress different lymphocytes. Accumulate in TME.

**Classes of rejection antigens**

- *Neoantigens*: Point mutations to existing binders/allow new proteins to bind to MHC
- *cancer-testis*: Usually expressed in male germ cells without MHC. Cancer can start expressing them, eg. *MAGE* (melanoma-associated antigens). ex: NY-ESO-1 (New York esophageal squamous cell carcinoma-1)
- *tissue differentiation antigen*: eg. CD19 B cell
- *overexpression antigen*: HER-2/neu
- *abnormal PTM*: underglycosylated mucin
- *skipped introns*:
- *viral oncogenes*: HPV in cervical carcinoma

**A look at melanoma specific antigens**

- tyrosinase (melanin production pathway) glycoproteins:
- gp100
- gp75
- MART1

Melanin is synthesized in special organelles called melanosomes and distributed across keratinocytes.

**Philadelphia chromosome** Piece of chrom 9 (ABL) breaks of and attaches to chrom 22 (BCR).

Bcr allows Abl (non receptor kinase) to bind to itself. Constitutively active molecule leads to growth / proliferation and *Chronic Myeloid Leukemia*.

You can then identify reactive T-cells to Bcr-Abl using tetramers

**Allografts** Expose donor lymphocyte infusion to leukemia specific peptides in-vivo for an increased leukemia killing + potentially less graft-vs-host response.

**Structure of a CAR**

ALL (acute lymphoid leukemia)

1/ CD19 2/ 3 ITAMs from zeta chain of CD3 3/ 4-1BB (TNF)

*Can achieve complete clinical remissions with ALL!*

**mABs used against cancer antigens**

- Competing with receptor ligand, preventing growth effect
- Recruiting immune response to large molecule bound to cell

*Herceptin* binding to *HER-2/neu* is a flagship example

Problems?

- Inefficient killing of cells after bound
- Hard to penetrate solid tumor
- Soluble antigens mopping up antibody

Different types of conjugation, either to mAB or just Fv fragment

- immunotoxins (usually a protein toxin): ricin A chain + Pseudomonas
- small molecule
- radioisotope
- ADEPT (antibody directed enzyme / pro drug therapy)

#### Cancer Vaccines

- 2005 clinical trial: HPV vacccines 100% effective in preventing cervical cancer
- Similar mechanism with liver cancer and hepatitis

#### Checkpoint blockade

- Trying to get tumor to express costims like B7 doesn't seem to work that well
- CTLA-4. Expressed on activated conventional T cell + T_regs. Competes with CD28 for B7 ligands. Ipilimumab
- PDL1. pembrolizumab.

## Vaccination

- variolation from corpse pustule in medical literature for 1000s of years
- Jenner - small pox vaccine. First to intentionally vaccinate with cowpox (-vacca)?
- Pasteur, extended name in his honor with chicken cholera vaccine

Most vaccines happen to be good because they stimulate neutralizing antibodies. But pathogens that require more complex response need better vaccines.

Specific examples:

1/ Malaria. Cycles between the liver and blood cells. Express different surface proteins. Hide inside cells. 2/ Tuberculosis. Hides inside macrophages. Can be sequestered with granulomas but create latent, living disease. 3/ HIV. Direct infection (and hiding) of T cell. High mutation. Latency.

#### Attenuated or killed

- *attenuated*: reduced pathogenicity, eg. genetically modified that continues into present for malaria (no vaccines currently)
- *killed*: vaccines can cause lethal responses, especially in immunosupressed

"Reverse immunogenetics" to identify candidate peptide antigens that activate innate sensors (eg. TLRs) as adjuvants

1/ Infections are insufficient to generate protective immunity:

- malaria
- tuberculosis
- HIV

2/ Cost, storage, deploying vaccines in developing countries difficult

#### Most vaccines generate antibodies

1/ Neutralize exotoxin produced by pathogen. Diphtheria + tetanus. 2/ Neutralize pathogen itself to prevent re-infection.

- Because of linked recognition, pathogens must have epitopes recognized by both T and B cells.

**Properties of effective vaccines**

- Safe. Some can die from toxin or catch disease.
- Work in large proportion of population.
- Long lived. Must prime B + T cells.
- Cheap.

Herd immunity is possible but with large percentages of vaccinations. For mumps, estimated to be @ 80%.

2004-2005 in UK saw uprise in mumps because MMR (mumps / measles / rubella) was swapped with just MR because of supply.

**Measles / Mumps**

Both *paramyxovirus* species Enter from respiratory droplets into resp. tract.

Measles: 1/ alveolar macrophages + dendritic cells -> lymph nodes -> T / B cells. 2/ *primary viremia* enters blood stream with T / B cells 3/ *secondary viremia* distributes to many organs

Mumps: More localized to parotid glands.

**Live attenuated viral vaccines**

Live viruses are more effective at recruiting active arms of immune system. Attenuation can be achieved by selection for growth in non-human cells. Pose risks to immunodeficient patients Recombinant viruses offer path forward

**Live attenuated bacteria vaccines**

Passaged BCG. Over 13 years + 200 passages to lose infectivity.

Malaria. Knockouts in sporozoites prevent transformation from liver to blood infection.

**Route of ingestion matters**

Injection is not only expensive and difficult at scale, it does not mimic natural route of entry.

Example: live attenuated polio developed by Sabin. Vaccine can also spread like normal virus in eg. fecal swimming pools.

Also, Proteins bind to epithelial cells and are resistant to proteases make good adjuvants:

- E. coli derived heat-labile toxin (HBT). Ex: introduce with oral tetanus toxin as adjuvant to build tolerance against lethal challenge.

Counter example: influenza. Antibodies developed in mucus and systemically for upper and lower respiratory respectively. Lower respiratory is what causes death. Oral ingestion would only prevent mild illness from upper resp.

**Bordetella pertussis**

Attenuated bacteria used to treat Whooping cough in early 1900s.

In 1972, Japan, whooping cough cases increased because of fear of encephalitis (brain swelling) pushed vaccination age from 3 months to 2 years.

Lead to development of acellular vaccine: single protein component isolated + purified. More effective and without side effects.

### Conjugate vaccines

T cell independent (TI) antigens stimulate B cells without T cell help.

Capsular polysaccharides (eg. meningococcus, pneumococcus) are such TI antigens and can be used on their own with adults.

However young children (<2) cannot mount TI response: immature marginal zone B cells (the cells that handle TI), signaling pathways maturing.

Cross link protein to carrier protein recognized by T cell.

### Adjuvants

- Adjuvant approved in specific vaccine use
- Alum (aluminum salt) is the only adjuvant approved.
- MF59 (oil-in-water emulsion)

Trigger TLRs, NOD-like receptors, NLRP3

### DNA vaccines

Stable and safe, but weak response. Encoding cytokines helps.

Trasnfecting directly in dendritic cells allows efficient presentation. Trials underway.

### Vaccines can be used for existing chronic infections

1/ Obvious immune response but fails to eliminate

- Helminth
- Tuberculosis 2/ Pathogen is "hiding"
- HIV
- herpes