Immunity and Autoimmunity

- MHC1 and MHC2
- Cells
  - Lymphocytes B & T
  - Antigen Presenting Cells
- Natural Killers
- Cytokines
Immunity and Autoimmunity

- Immunity at work
- Innate Immunity
- Adaptive Immunity
- Slowing Autoimmunity; How
Immunity and Autoimmunity

- One Case of autoimmunity,
- How you should change your Practice to take into account The Case
- Credits
MHC Molecules

- Major Histocompatibility Complex (MHC) or Human Leukocyte Antigens (HLA)
MHC1

- MHC1
- 4 subunits transmembrane
- Chromosome 6
- On all nucleated cells, not RBCs
- D/B/A HLA A B and C eg HLA B27
MHC 1

- Peptide antigens presented in the groove
- The peptide antigens come from internal breakdown of proteins, either exogenous or endogenous
- The latter can be from normal cell metabolism
MHC2

- MHC2: the ‘Pro’ molecule
- 4 subunits, also transmembrane
- Also Chromosome 6
- On all Antigen Presenting Cells or ‘Professional’ APCs
- D/B/A HLA DM, DO, DP, DQ, DR
MHC2

- Peptide antigens in the groove
- 8-10 amino acid peptides in groove
- Antigens come from extracellular proteins
- Secretion similar to MHC1 but involves an endosome and lysosome path
Peptide

MHC 2

Membrane
Cellular Actors

- T Cells
  - 1 in $10^5$ usually; can activate and clonally expand to 10-20% of all lymphocytes
- B Cells
- Natural Killer cells
- Antigen presenting cells
T Cells

- T cells selected in and mature in Thymus
- T cell receptors define T cells as does CD3
  - Bind to MHC1/2 molecules attached to peptides
- Positive selection leads to T cells with intermediate affinity for an MHC class molecule
- Negative selection removes T cells that recognize ‘self’ peptides
- Thymus shrinks 3% yearly throughout middle age
T Cells

- Heterogeneous binding due to recombination
- Multiple Types and Subtypes of both B and T cells; discuss 2 major T cell types and no subtypes
- CD4 cells or Helper cells
  - CD# : Cluster of Differentiation, ‘do engineered monoclonals bind to the surface molecule?’
T Cells

- CD4 a transmembrane protein (like MHC molecules and many we discuss today) associated with the T cell receptor
- Many transmembrane proteins signal in the cytoplasm
- Signaling happens due to conformational changes in the extracellular protein structure leading to the same intracellularly
MHC 2 and Helpers

- T Cell receptor of CD4+ cells bind to $\alpha_1 \beta_1$ subunits of MHC2
- CD4 binds to the MHC2 $\beta_2$ Subunit
  - Therefore Helper cells are MHC class 2 restricted
- CD4 can signal in the cytoplasm
Intracellular space (APC)

Extracellular space

Intracellular space (Lymphocyte T)

CD4 molecule

MHC2

Peptide

Lymphocyte T receptor

Lck

<--Signaling portion of complex
MHC2 Helper T cells

- Why attach to antigen presenting cells and check the peptides
- To signal the Helper cell that ‘not same’ has been found and what it is and activate the Helper cell so they can help
- But not yet
T Cells

- CD 8 Cells or Cytotoxic T cells
- T cell receptor binds to $\alpha_1\alpha_2$ of MHC 1 (on all nucleated cells) and the peptide groove and checks the 8-10 amino acid residue for sameness
- CD8 from CTLs binds to $\alpha_3$ subunit of MHC1
- MHC1 class restricts these T cells
MHC I

- Not same, then cell immediate immunity (activation) and apoptosis of the cell which presented the peptide
B Cells

- B cells mature in the Bone marrow (Bursa of Fabricius in birds)

- B cell receptors which in mature B cells are Immunoglobulins D or a monomer of M plus an associated molecule CD79AB

- Bind to antigen and become activated
  - Can be much more specific than T cell binding
  - Activation leads to Ig* production and possible presentation of the antigen to T cells for activation
B Cells

B cell Receptor

CD79A

CD79B

Immunoglobulin (Antibody)

Signal propagated through CD79 tail
B Cells

- During maturation negative selection for self
  - If self binding is strong:
    - clonal deletion
    - receptor editing
    - anergy
B Cells

- Can recognize foreign substances (peptides, glycoproteins, polypeptides, entire viruses/bacteria)
- T cells only recognize short peptides
- Activation can occur with or without T Helper cells; when with - slower to start and more affinity
B Cells

- First antibody production is generally IgM
- Switches to (IgG, A, E) later
- Isotype switching is another signaling issue we will not discuss
Antigen Presenting Cells

- ‘Professional’ APCs, Macrophages, Monocytes, B cells, Dendritic cells and Langerhans Cells
- MHC 2 are the presenting molecules after the cells digest foreign proteins and MHC 2 migrates to the cell surface
Immunoperoxidase stain of m. Ulcerans in Dendritic cells/Langerhans cells
Natural Killer Cells

- Not T cells and not Killer T cells
- Mature in Lymph tissue generally
- Have Dominant MHC1 receptors on their surfaces that suppress their activation
- No T cell antigen receptors
- Release granzymes that can cause osmotic lysis or apoptosis
Natural Killer Cells

- Major part of the innate immune system
- Do not require adaptation to the invader
- Job is to recognize infected or tumor cells...
Natural Killer Cells

- ‘Missing Self’ hypothesis
- Sick and Malignant cells have a low density of MHC1 molecules on their surfaces
- When NKs come in contact with an ill cell the inhibitory MHC1 receptors do not get much of a (-)signal but they get activation signals from the ill cell
Natural Killer Cells

- Also release TNFα, IFγ and IL 10, the latter being anti-inflammatory.
- Interacts with Adaptive immunity through cytokines.
- Cell lysis occurs with non-infected cells and apoptosis which destroys the cell content in infected cells.
Cytokines

- Cell Signals Not Hormones
- Interferons, Tumor Necrosis Factors, Interleukins, Lymphokines
- Autocrine, paracrine, endocrine (e.g., pyrogens)
- Not secreted from one cell type as are hormones
- Picomolar vs nanomolar (hormones) that increase 1000 fold vs several fold (TSH)
Cytokines

- Duality of action: maybe pro and/or anti-inflammatory
- Pleiotropy of action: different effects different sites
- As a treatment intervention, therefore have to be tested extensively on humans
Cytokines

- Result from activation of B/T cells, APCs, Natural killer cells
- Provide an extra signal for the action of these cells both activation and deactivation
Chemokine's

- Chemotactic cytokines
  - Small molecules that help:
    - Maintain homeostasis
    - Attract White blood cells to inflammatory areas
Cellular adhesion Molecules

- Also result from Cell activation
- Integrins also affect platelet adhesion (collagen, thrombin)
- Cadherins
- Selectins
Innate Immunity

- Fast: immediate vs 2-3 days
- Innate immunity is hardwired to recognize foreign antigens that are most likely associated with invaders
- Pattern Recognition Receptors (PRRs) to detect Pathogen Associated Molecular Patterns (PAMPs)
- PAMPs are evolutionarily conserved
- PRRs are as well
Innate Immunity

- PAMP examples
  - Mannose in somatic cells but soluble or hidden
  - Mannan in yeast = mannose polysaccharide $\alpha_1$-6 backbone; $\alpha_1$-2 and $\alpha_1$-3 branches. Starch is $\alpha_1$-4 glucose with $\alpha_1$-6 branches
  - Flagellin
  - LPS Sepsis/Shock
Innate Immunity

- PRR Example: Toll like receptors
- Toll Gene Establishes Dorsal Ventral structure in Drosophila
- Present in plants invertebrates through mammals
- On Sentinel Cells: Dendritic cells and Macrophages
- How important are PRRs??
Innate Immunity

- Intracellular Effector Pathways
- Inflammation, Adaptive immunity
- stimulus, Apoptosis
Extracellular

Toll-like Receptors
- Bacterial Cell Wall Compounds
- Peptidoglycans
- Lipopeptides
- Lipopolysaccharides
- Flagellin
- Viruses
- Mycobacteria
- N-linked Mannan
- Zymosan
- Fungal Hyphae
- N-linked Mannan

C-type Lectin Receptors
- SAP130
- d Mannose
- Cord Factor
- dsDNA (Viral Infection, Bacteria)

Viral Infection
- Viral RNA
- Viral DNA

DNA Sensors
- RNA poly III Ca2+
- MRE11
- LRRP1
- DDX41
- IFI16
- AIM2
- DHX15

RNA Sensors
- RIG-I
- MDA5
- LSU2
- LSm4A

Mitochondria
- ER
- STING
- PKR
- MAVS

Inflammasomes
- NLRP1
- NLRP3
- NLRP6
- NLRC4
- RIG-I
- AIM2

Caspase-1 (Caspase-6)
- Asc

Pro-inflammatory Cytokines & Chemokines
- Type I IFNs

Cytoplasm

Nucleus

Inflammation, Immune Regulation, Autophagy, Survival, Proliferation, Apoptosis, Necrosis/Necroptosis
Adaptive Immunity in Action
Simplistically

1. Binding to antigen
2. Chemical signal
3. Becomes plasma cell
4. Releases antibodies
Recall that I said 'not yet' for activation earlier when an APC MHC2 attaches to the correct T cell TCR molecule. Well, it requires TWO SIGNALS not just the TCR-MHC peptide recognition. Costimulation requires 2 or more signals.
Arachidonic acid metabolites and inflammation

Cell membrane phospholipids → Phospholipases → ARACHIDONIC ACID

- Cyclooxygenase:
  - Prostaglandin G₂ (PGG₂)
  - Prostaglandin H₂ (PGH₂)
  - Prostacyclin (PGI₂): Causes vasodilation, inhibits platelet aggregation
  - Thromboxane A₂ (TXA₂): Causes vasoconstriction, promotes platelet aggregation
  - PGD₂
  - PGE₂: Vasodilation, Increased vascular permeability

- 5-Lipoxygenase:
  - 5-HPETE → 5-HETE: Chemotaxis

- 12-Lipoxygenase:
  - Leukotriene A₄ (LTA₄)
  - Leukotriene C₄ (LTC₄)
  - Leukotriene D₄ (LTD₄)
  - Leukotriene E₄ (LTE₄)
  - Vasoconstriction, Bronchoospasm, Increased vascular permeability

COX-1 and COX-2 inhibitors, aspirin, indomethacin inhibit

Steroids inhibit

Immunity

- You can make an immune system 😎

- With what you know now you can see that one has to activate B cells (antibody production) and T cells (cytotoxicity, helper B cell function) in a specific fashion, through recognition pathways, to have adaptive immunity can be aided by arms of innate immunity
Biologics for Autoimmune disorders

- It's easy

- Find out what sets off the activation

- Sorry, only 'known' for a few things

- Not clear that removing the activator will stop the activation although it should

- Right???
Biologics for Autoimmune Disorders

- Turn off the activator (main switch)
- Lyme disease, Reactive arthritis: early antibiotics
- Rubella
- Over time these frequently fade out
- Gonorrhea a special case
Biologics for Autoimmune Disorders

• It appears that in many illnesses (RA, SLE, Myositis, MS) the main breaker switch is left on

• We do not know the switch

• So we’ll just have to turn off some of the switches down the line until we find the original stimulus to interrupt
Biologics for Autoimmune Disorders

- Well, we can do that
- Target enzymes in the cytoplasm cascade
  - MAP Kinase failed in RA
  - Janus Kinase has not
    - JAK inhibitors for malignancies and RA
Biologics for Autoimmune Disorders

- We can target signaling
  - Cytokines are a first target in rheumatology
  - Surface signaling molecules are another
  - Adhesion molecules
Biologics for Autoimmune Disorders

- Cytokine Blockers
- They do not always work
- They have significant side effects
Biologics for Autoimmune Disorders

- Cytokine Blockers
- Side effects include
  - Psoriasis from TNF Blockers
  - They fail after time
    - Antibodies to the antibodies
    - The Body's attempt at homeostasis—work around the blockade
Table 1. Examples of antibodies tested for the treatment of arthritis and other inflammatory/autoimmune diseases.

<table>
<thead>
<tr>
<th>Target</th>
<th>Agent</th>
<th>Type</th>
<th>Mechanism of Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1</td>
<td>Anakinra a</td>
<td>Recombinant IL-1Rα</td>
<td>Prevents binding of IL-1β to IL-1Rα</td>
</tr>
<tr>
<td></td>
<td>Anti-IL-1β c</td>
<td>Human IgG1 monoclonal antibody (mAb)</td>
<td>Binds tightly to IL-1β and neutralizes it</td>
</tr>
<tr>
<td></td>
<td>Canakinumab b</td>
<td>Human IgG1 mAb</td>
<td>Neutralizes the activity of IL-1β</td>
</tr>
<tr>
<td></td>
<td>Gevokizumab b</td>
<td>Humanized IgG2 mAb</td>
<td>Neutralizes the activity of IL-1β</td>
</tr>
<tr>
<td></td>
<td>LY2189102 b</td>
<td>Humanized IgG4 mAb</td>
<td>Neutralizes the activity of IL-1β</td>
</tr>
<tr>
<td></td>
<td>Rilonacept b</td>
<td>Ligand-binding domain of IL-1RI and human IgG1 fusion protein</td>
<td>Attaches to and neutralizes circulating IL-1β before it can bind its receptor</td>
</tr>
<tr>
<td>IL-6</td>
<td>MAB406 c</td>
<td>mAb</td>
<td>Blocks IL-6 signaling</td>
</tr>
<tr>
<td></td>
<td>MRA b</td>
<td>Humanized mAb</td>
<td>Inhibits IL-6 signaling</td>
</tr>
<tr>
<td></td>
<td>Tocilizumab a</td>
<td>Humanized mAb</td>
<td>Binds to IL-6Ra chain and blocks IL-6 signaling</td>
</tr>
<tr>
<td>TNFα</td>
<td>Infliximab a</td>
<td>Recombinant IgG1 mAb</td>
<td>Binds to TNFα and prevents it from binding to its receptor</td>
</tr>
<tr>
<td></td>
<td>Adalimumab a</td>
<td>Recombinant IgG1 mAb</td>
<td>Binds to TNFα and prevents it from activating TNF receptors</td>
</tr>
<tr>
<td></td>
<td>Etanercept a</td>
<td>Extracellular domain of TNF receptor II (p75) and the Fc portion of IgG1 fusion protein</td>
<td>Functions as a decoy receptor to TNFα</td>
</tr>
<tr>
<td></td>
<td>Golimumab b</td>
<td>Human mAb</td>
<td>Neutralizes TNFα bioactivity</td>
</tr>
<tr>
<td>IL-17</td>
<td>Secukinumab b</td>
<td>Human IgG1k mAb</td>
<td>Selectively binds and neutralizes IL-17A</td>
</tr>
<tr>
<td></td>
<td>Ixekizumab b</td>
<td>Humanized IgG4 mAb</td>
<td>Neutralizes IL-17A</td>
</tr>
<tr>
<td></td>
<td>Brodalumab b</td>
<td>Human anti-IL17RA mAb</td>
<td>Inhibits the activity of IL-17</td>
</tr>
<tr>
<td>IL-12/IL-23</td>
<td>Ustekinumab b</td>
<td>Human IgG1k mAb</td>
<td>Blocks the biologic activity of IL-12 and IL-23 through their common p40 subunit by inhibiting their receptors</td>
</tr>
<tr>
<td></td>
<td>Guselkumab b</td>
<td>Human mAb</td>
<td>An IL-23p19-targeted mAb</td>
</tr>
</tbody>
</table>
Biologics for Autoimmune disorders

- Multiple Sclerosis/Crohn's: Tysabri (Natalizumab) blocks $\alpha_4$ integrin necessary for white cells to cross the endothelium into organs.

- Ulcerative Colitis: Entyvio blocks $\alpha_4/\beta_7$ integrin, gut specific Peyers patches for UC.
Biologics for Autoimmune disorders

- Block the Costimulus Pathway
- Both Sides of the coin with CTLA4
  - Cytotoxic Lymphocyte Associated protein 4
  - Down regulator
  - Binds to CD80/86 and inhibits T Cell activation
  - Made in Activated T cells serves as feedback control
Biologics for Autoimmune disorders

- CTLA4 Replaces CD28 in binding to CD28
- 1. If we block Binding of CD80/86 to CD28 we reduce Activation
- 2. If we block CTLA4 we maintain activation
- So we’ve done just that 1 for RA and 2. for Melanoma
Abatacept modulates the immune response by binding to CD80/CD86 on an antigen-presenting cell (APC), such as a dendritic cell, thus preventing costimulatory binding of CD28 on naive T cells and attenuating T-cell activation.

Abatacept/Orencia
Ipilimumab
What about Smoking?

- As smoking rate decreased in this country so did RA
  hmmmmm
- Smokers get more RA
- Smokers have worse RA
- Smokers are less likely to benefit from our interventions
What about Smoking?

- First degree relatives of RA patients without diagnosed RA are 1.6x (1.09-2.32) times as likely to have swollen joints if smokers than if nonsmokers.
- Half of RA pts have IgM RF or Anti CCP antibodies years before RA (up to ten).
What about Smoking?

- Although smoking appears to reduce immune function generally in the lung it also increases the production of PeptidylArginine Deiminase (PAD) which removes one of the terminal amines from a polypeptide/protein and creates citrulline or in these cases citrullinated proteins/peptides.

- These citrullinated proteins may have a different structure than native proteins and can be seen as ‘Not Self’ eg citrullinated Vimentin.
What about Smoking?

- Some first degree relatives (FDRs) of RA pts have ACPA and RF antibodies in sputum.
- On HRCT abnormalities c/w Inflammatory dz were seen in pts with RF and ACPA antibodies.
What About the Gut and the Teeth

- For you GI Flora aficionados:
  - Similar stories are being constructed for the gut flora and severe periodontitis
What about Smoking?

- So we see a strong connection but we have not yet fully defined how a local mucosal inflammation causes the immune system to lose tolerance (which we did not discuss today) and become the systemic illness Rheumatoid Arthritis (or SLE)
And What About Smoking

- Are you going to add RA to the bad results from smoking??
“Finally no NSAIDs in Dengue”

–Dr H