Fundamentals of Immunology

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BIOC 372x Study Guide

This study guide covers Part Two: Cellular Immunity and Signaling

Contents

Lecture L08- The Major Histocompatibility Complex
Lecture L09- Antigen Processing and Presentation
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Lecture L13- Inflammation and Extravasation
Lecture L014- Cellular Immunity

Glossary

Image Attribution

The Part One Study Guide covers:

Lecture L01- Introducing the Metaphors
Lecture L02- Surveying the Cells & Organs of the Immune System
Lecture L03- Innate Immunity
Lecture L04- Antigens & Antibodies
Lecture L05- Organization & Expression of Immunoglobulin Genes
Lecture L06- Development of B Cells
Lecture L07- Complement
Reference figure: Labeled IgG Antibody Model

This figure summarizes information on antibody construction covered in Part One. We hope this provides you a handy reference to correlate features of antibody structure, function and synthesis with those of T-cell Receptors.

Left side labels: Antibody Structure

- Internal color-coded lines indicate peptide backbone.
- Sequins (silver dots) indicate disulfide bonds.

Right side labels: Source of information coding for structures

- Indicates what gene regions supplied the information for the amino acid sequence as well as the locations of gene rearrangement joins, splicing joints and leader peptide removal.
Lecture 8

The Major Histocompatibility Complex

“There’s only one person in the whole world like you. …… There’s never been anybody exactly like you before, and there will never be anybody exactly like you in the future. You’re the only one.” Fred Rogers, 1928 - 2003

I. Basic Characteristics: used to hold antigen for T cells, determine transplantation success.

A. Genes: human HLA complex on chromosome 6, organized into 3 regions:

1. Class I MHC
   a. Codes for a three-domain protein.
   b. The main peptide (α) associates with a second (β), which is coded for on an entirely different chromosome (# 15).
   c. Displays antigen (small peptide) to T<sub>C</sub> (cytotoxic) cells.
   d. Everyone has a block of 3 different MHCI alleles from Mom and 3 from Dad (A,B & C below)
   e. The genes are wildly polymorphic: each one of these 6 could be any one of as many as 100 different alleles.
   f. Expressed by almost all nucleated cells, but not found on red blood cells, sperm or nerve cells.
2. Class II MHC
   a. Each gene codes for 2 separate peptides (α and β) which function together.
   b. present antigen (small peptide) at the end between α and β to TH (helper) cells
   c. Everyone has 3 of these genes from Mom and 3 from Dad.
   d. block of genes on the centromere end (DP, DQ and DR)
   e. wildly polymorphic: each one of these 6 could be any one of as many as 100 different alleles.
   f. expressed by antigen presenting cells (macrophages, dendritic cells, B cells)

3. Class III MHC
   a. completely different - generally not membrane components
   b. code for secreted proteins involved in the innate responses: complement proteins, cytokines, and heat shock proteins.
   c. included because the genes just happens to lie between I and II genes.

B. Haplotypes

1. Both Class I and Class II genes are tightly linked, with a crossover rate of about 0.5%. This means that any one individual tends to pass on the collection of 3 class I and II MHC from Mom as a unit and the collection of 3 class I and II MHC genes from Dad as a unit.
2. This unit or block is called a haplotype.

3. Let’s look at a marriage in which one partner has two different blocks of these genes, haplotype A from her Mom and haplotype D from her Dad and marries someone with a completely different block, M from Mom and P from his Papa.

![Figure 8-3, Locus Heritability]

4. These blocks can combine to produce AM, AP, DM and DP, illustrated here as four possible genotypes in the children.

5. This is why, when people discuss the possibility of tissue transplants from a sibling, they say you have a one-in-four chance of a match.

C. Recombinant Haplotypes

1. Crossing over can occur during meiosis of the germ cells of either Mom or Dad.

2. Rarely, if the crossover happens between the I and II blocks, this produces a recombinant haplotype.
3. If a sperm with one of these joins and fertilizes an egg, the resulting child is highly unlike to EVER have a sib match – that would require the exact same crossover event, the same choice of one of the two crossover results and a union with the exact same egg haplotype.

D. Inbred Mice

1. If you inbreed mice with a series of brother-sister matings, you can produce different strains in which each mouse is histocompatible with the others.

2. This is because each mouse of a given strain will have the same haplotype on each homologous chromosome.

3. Different strains will have different haplotypes.

4. If you then take two different inbred strains and cross them you get an F₁ that:
   a. can accept grafts from any other F₁ (same types).
   b. can accept graft from either parental strain (doesn't graft on any non-self).
   c. cannot donate to either parent (will provide other parent's antigen).
E. Practice

1. Let's look at those mice again.
   a. How many different MHC I molecules can left hand (yellow) strain make? MHC II
   b. How many different MHC I molecules can the right hand (peach) strain make? MHC II?
   c. How many different MHC I molecules can the F1 progeny make? MHC II?

2. Here's the maternal and paternal haplotypes of an individual from an isolated village in remote area.
   a. The MHC I A haplotypes are the same from Mom and Dad, although MHC I B and I C are different.
   b. The MHC II DP αβs are different but the MHC II DQs and DPs are identical.
Figure 8-6  Human genotype showing some inbreeding

3. How does this influence variability of receptors?
   a. How many different MHC I molecules can this person make?
   b. How many different MHC II molecules can this person make?
   c. This person leaves the village for the city and married someone completely unrelated who shares not alleles for either MHC. How many different MHC I molecules will their child make? MHC II?

At this point you can try the supplementary problems in the courseware section for this lecture.

Figure 8-7, Foam board models of MHCI (left) and MHC II (right)
II. MHC Protein Structure - Both kinds are membrane-bound glycoproteins with common structural elements, including a peptide-binding cleft.

A. Class I MHC

1. \( \alpha \) chain
   a. 3 major domains, \( \alpha_1, \alpha_2, \alpha_3 \) (amino to carboxyl)
   b. peptide binding site between \( \alpha_1 \) and \( \alpha_2 \)
   c. \( \alpha_3 \) connected to
   d. transmembrane segment with cytoplasmic tail

2. \( \beta \) microglobulin chain
   a. gene actually located on a different chromosome, #15
   b. peptide associates with \( \alpha_1 \) by weak linkages
   c. necessary for membrane expression of whole molecule
   d. This does not have a membrane-spanning region, but rather attached by weak bonds to the domain of the class I MHC immediately exterior to the membrane.

3. Homologies
   a. \( \alpha_3 \) domain and \( \beta \) chain resemble each other.
   b. Both resemble the constant domains of immunoglobulins.
   c. Note the "bread and butter" sandwich structure.
   d. Gene transcript requires processing (no alternative splicing forms), but the DNA is not changed.

4. Interactions
   a. \( \alpha_1 \) and \( \alpha_2 \) form a platform structure with 8 antiparallel \( \beta \) strands connecting two helical regions.
b. The space between the helices forms a deep groove, the peptide-binding cleft.

c. Long enough to hold a peptide of 8 to 10 amino acids.

d. The platform region also interacts with the \( \beta \) microglobulin, which kind of supports one side of the structure.

e. The \( \beta \) peptide is necessary for the proper folding of the \( \alpha \) peptide and its placement into the cell membrane.

B. Class II MHC

1. \( \alpha \) chain
   a. two major external domains (\( \alpha 1 \) and \( \alpha 2 \))
   b. transmembrane domain with cytoplasmic tail

2. \( \beta \) chain
   a. two major external domains (\( \beta 1 \) and \( \beta 2 \))
   b. transmembrane domain with cytoplasmic tail

3. Homologies
   a. \( \alpha 2 \) and \( \beta 2 \) resemble the class I MHC \( \alpha 3 \) domain and \( \beta \) chain and immunoglobulin constant regions.
   b. \( \alpha 1 \) and \( \beta 1 \) resemble the class I MHC \( \alpha 2 \) and \( \alpha 1 \) domains respectively.
   c. MHCs are also in immunoglobulin superfamily.
   d. Gene transcript requires processing (no alternative splicing forms), but the DNA is not changed.

4. Interaction
   a. The \( \alpha \) and \( \beta \) dimers are joined by weak interactions.
   b. The antigen-binding cleft is formed by the \( \alpha 1 \) and \( \beta 1 \) interaction.
III. Specifics of Peptide Binding

Class I and II molecules both bind peptide hydrolysis products of proteins, the peptides being inserted into their respective cleft regions.

A. Common Elements

1. The peptides produced by hydrolysis may come from any part of the molecule.

2. Peptides are extended in the cleft, and do not have their native secondary or tertiary structure.

3. MHC molecules can bind a variety of peptides, although each MHC has some (broad or promiscuous) specificity.

B. Class I MHC

1. Sides of the cleft defined by α helices

2. Bottom of the cleft define by β sheets

3. Ends of the cleft also defined.


   a. 8 or 10 can fit, because of bending.

   b. Peptide bows outward slightly,
c. Bowing helps display middle of the peptide from out of the groove of the cleft.

5. Peptides held on the ends by their anchor residues, which interact with specific side chains of the amino acids of the class I MHC.
   a. Carboxy (COOH) terminal anchor (amino acid #9 of the peptide) is typically hydrophobic.
   b. Amino acid # 8 and even # 7 may be involved in anchoring.
   c. Amino terminal anchor is #2.
   d. Middle amino acids may vary, although exactly what they are will be important to the T cell receptor they will ultimately interact with.

C. Class II MHC
   1. Sides of the cleft defined by \( \alpha \) helices
   2. Bottom of the cleft defined by \( \beta \) sheets
   3. Ends of the cleft are **undefined**: the peptide can stick out like a long hotdog in a short bun.
   4. Peptides bound have 13 to 18 residues, but only 13 of them fit the cleft.
   5. Peptides do not bow outward, but rather lie flat in the cleft.
   6. Peptides therefore interact with the class II MHC molecule at a series of places in the cleft region.
   7. Different MHCs will have different binding specificities based on these interactions.
   8. The MHC amino acids involved in the interaction show the most of the polymorphism of the molecule.
IV. Genetic Expression

A. Polymorphism and Individual Expression

1. There are an enormous number of different possible alleles coding for different versions of all the class I and II MHC classic genes and a fair amount of variety in the non-classic ones as well.

   a. For HLA I in humans, we have identified 60 versions of A, 110 versions of B, and 40 versions of C.

   b. This underestimates the diversity, because we usually test whoever is hand, and that means disproportionately from people of European descent.

2. Any one individual, however, will express only those alleles present in his genotype.

3. This means that any one individual will express 6 (3 maternal 3 paternal) class I MHC alleles, 6 (3 maternal 3 paternal) class II MHC alleles.

4. The 12 classical alleles come from this incredible assortment of possibilities, meaning that any two unrelated individuals are very unlikely to share them.

5. Although it does happen.

B. Cellular Expression

1. Class I MHCs are expressed by most cells, but to varying degrees:

   a. Very high levels on lymphocytes - critical in presenting

   b. Low on fibroblasts, muscle cells, and liver cells

   c. None on nerve cells, red blood cells or sperm

2. Class I MHCs present antigen from normal turnover of interior proteins.

   a. They will bind and present peptides from cytochromes, histones, ribosomal proteins, and indeed any cell protein, cytoplasmic, nuclear, or mitochondrial.

   b. These proteins will be recognized as self-proteins early in development.
3. Class I MHCs present antigen from turnover of abnormal interior proteins as well.
   a. Viral proteins degraded into peptides will also get bound to some of the class I MHC and presented.
   b. These will be recognized as non-self and trigger an immune response, resulting in attack by T<sub>C</sub> cells.

4. Class II MHCs are not expressed by most cells.
   a. Expressed by antigen presenting cells, B cells, macrophages, and dendritic cells.
   b. Expressed upon induction by thymic epithelial cells
   c. Epithelial cells and a few other types responsive to certain cytokines
   d. Present exogenous antigen to T<sub>H</sub> cells, more in next lecture.

<table>
<thead>
<tr>
<th>Table 8-1 MHC Constituents of Various Cell types</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neither MHC I nor II</td>
</tr>
<tr>
<td>red blood cells</td>
</tr>
<tr>
<td>sperm cells</td>
</tr>
</tbody>
</table>

Figure 8-9, Representation of maximum possible variety on the surface of a presenting cell
C. Expressing the complete genotype

1. Considering the class I MHC, the chances are that all 6 alleles, three from Mom and 3 from Dad, will be different.
   
a. This will code for 6 different surface binding molecules, each with a different specificity for peptide binding.
   
b. What about the β microglobulin? This is coded for by a different gene on a different chromosome (15), and may well have different alleles as well.

2. Consider class II MHC, multiple figure
   
a. You have three α from Mom and three α from Dad.
   
b. You have three β from Mom and three β from Dad.
   
c. However, any DR α from either parent may combine with any DR β, resulting in 4 different DR combinations.
   
d. This is also true for DP and DQ, so there are 12 different MHCII combinations, assuming no inbreeding.
   
e. This broadens the possibilities for effective presentation to T\textsubscript{H} cells.

D. Comparing MHC Molecules and Lymphocyte Receptors

1. All cells in any one individual have the same genetic possibilities for making either kind of MHC.

2. The genes for MHCs do not rearrange, nor do they generate different splicing isoforms.

3. The extreme specificity of the response to the antigen presented is a property of the T-cell receptor, not the MHC presenter.

4. Description of the complex figure show different combinations of proteins:

V. But Wait! There’s More! Non-classical Alleles

A. Non-Classical MHC Genes – (more detailed map at the end of the handout)

1. Interspersed in the Class II MHC genes are genes that code for elements of the system that processes antigen for Class I MHC presentation (go figure.)
2. Interspersed in the Class I MHC genes are those for "non-classical" membrane proteins involved in a variety of specialized receptor, transport, or presentation functions.

3. Most have the same general three-domain shape as MHC I, associate with β microglobulin, and are typically named HLA – something, for Human Leukocyte Antigen.

B. CD1 and Lipid Presentation

1. T cells also recognize glycolipids produced by hydrolysis of bacteria.

2. Presenting cells add these lipids to special MHC molecules, loading them in the phagolysosome.

3. The binding part looks a lot like a class I MHC and also uses the same β microglobulin.

4. The lipid-binding MHCs come in 5 versions, CD1A-E, all coded for on Chromosome 1.

Figure 8-10, Comparable representations of MHC II (right), MHC I (middle) and CD1 (left, holding lipid antigen)
Table 8-2 Compare and Contrast Properties of the Class I and II and CD1 MHCs

<table>
<thead>
<tr>
<th>MHC I</th>
<th>MHC II</th>
<th>CD (A-E)</th>
</tr>
</thead>
<tbody>
<tr>
<td>displays endogenous</td>
<td>presents exogenous</td>
<td>displays exogenous lipid</td>
</tr>
<tr>
<td>peptide</td>
<td>peptide</td>
<td></td>
</tr>
<tr>
<td>to T&lt;sub&gt;c&lt;/sub&gt; cells</td>
<td>to T&lt;sub&gt;H&lt;/sub&gt; cells</td>
<td>to T&lt;sub&gt;H&lt;/sub&gt; cells</td>
</tr>
<tr>
<td>on most nucleated cells</td>
<td>on presenting cells</td>
<td>on presenting cells</td>
</tr>
<tr>
<td>1 peptide, 3 domains</td>
<td>2 peptides, 4 domains</td>
<td>1 peptide, 3 domains</td>
</tr>
<tr>
<td>chromosome 6</td>
<td>chromosome 6</td>
<td>chromosome 1</td>
</tr>
<tr>
<td>plus β globulin(c-some 15)</td>
<td>plus β globulin (c-some 15)</td>
<td></td>
</tr>
<tr>
<td>closed cleft between α1 and α2</td>
<td>open cleft between α1 and β</td>
<td>deep cleft between α1 and α2</td>
</tr>
<tr>
<td>loaded in RER</td>
<td>loaded phagolysosome</td>
<td>loaded in phagolysosome</td>
</tr>
</tbody>
</table>

VI. MHC and Disease

A. MHC and Immunity

1. Determinant Selection: While MHC binding is promiscuous, it is not universal. Some MHCs bind some antigens better than others, and some antigens do not seem to get displayed properly.

   a. The perils of inbreeding: Cheetahs are at risk of extinction in part because they are so inbred.

   b. Disease as an evolutionary selective force: Populations constantly endure a sweep of infection that kills off a substantial fraction of its members. Those remaining will usually have a statistically significant difference in the distribution of MHC (and other) alleles.

Figure 8-11, cheetah
Figure 8-12 Map of MHC (HLA) locus
2. HIV “elite controllers” – there are people who have contracted HIV, but maintain low levels of the virus and stay healthy for decades.
   a. Studies comparing their DNA to those of susceptible people showed a number of common snips in the DNA of chromosome 6.
   b. Upon analysis, the relevant region was in the gene coding for MHC I, HLA-B, specifically the part determining the binding groove. Variant #27 is associated with slow progression.
   c. Thus “elite controllers” are particularly effective at binding and displaying HIV-derived peptides and thus very effective at activating Tc cells.
   d. The Tc cells, in turn, keep the viral levels so low that the T\textsubscript{H} cells remain protected and thus able to continue coordinating the immune response.
   e. However, the bad news is that #27 is also associated with increased risk of ankylosing spondylitis, an autoimmune disease which results in fusion of the spinal cord vertebrae.

B. MHC and Susceptibility

1. Possession of a particular allele increases your relative risk, sometimes very substantially.

2. For example, a variant of DR2, that if you have it you are 130 times more likely to exhibit narcolepsy as a typical member of the general public. Narcolepsy is now classified as an autoimmune disorder.

3. On the other hand, possession of a particular allele does not predictably doom you to a certain disease; environment and dumb luck also play a role.

4. An exception is hereditary hemochromatosis, which results from having two copies of a mutant variant of HLA- HFe.

5. This codes for a surface protein on the cells of the digestive tract, and when it’s there, it interferes with negative regulation of iron uptake.

Some Extras:

Video: Rusty, the narcoleptic dog, falls asleep every time he get excited. [http://www.youtube.com/watch?v=jTj3a2nHw8k](http://www.youtube.com/watch?v=jTj3a2nHw8k)


Lecture 9

Antigen Processing and Presentation

I. T Cell Antigen Recognition

A. Common Elements of T\textsubscript{C} and T\textsubscript{H} Cell Recognition

1. T cells only recognize peptide antigens not native proteins.

2. These peptides must be attached to some kind of MHC molecule extending from the surface of a cell.

3. The T-cell receptors recognize the combination of self-MHC and antigen.

B. Specific T cells

1. T\textsubscript{C} Cells

   a. Cells have CD8 assisting the receptor.
   b. Cells bind to Class I MHC plus antigen.
   c. Antigen \textit{displayed} is endogenous - arises from proteins produced by the cell.
   d. Most cells display antigen on MHC I.
   e. T\textsubscript{C} cells respond by attacking the cells displaying abnormal antigens.
   f. Thus the cells displaying foreign antigen are target cells.

Figure 9-1 CD8/ MHC I

Figure 9-2 CD4/ MHC II
2. Specific to \( \text{T}_\text{H} \) Cells

   a. \( \text{T}_\text{H} \) cells have CD4 assisting the receptor.
   b. \( \text{T}_\text{H} \) cells bind to Class II MHC plus antigen.
   c. Antigen **presented** is exogenous - arises from proteins hydrolyzed after phagocytosis or endocytosis.
   d. B cells, macrophages or sentinel dendritic cells do most of the presenting, and are called professional antigen presenting cells.
   e. \( \text{T}_\text{H} \) cells respond by producing cytokines and other signals that stimulate a variety of immune responses.

C. Professional Antigen Presenting Cells (APCs) - the most important source of antigens for \( \text{T}_\text{H} \) cells.

1. sentinel (but not follicular) dendritic cells

   a. most effective
   b. constitutively express class II MHC and costimulatory (B7) molecules
   c. can activate naïve \( \text{T}_\text{H} \) cells

2. macrophages

   a. activated by phagocytosis
   b. express class II MHC and costimulatory (B7) molecules after activation.

3. B cells

   a. constitutively express class II MHC
   b. express costimulatory (B7) molecules after activation (receptor cross-linked by antigen)
   c. most effective at activating \( \text{T}_\text{H} \) cells with small amounts of antigen

Figure 9-3 Langerhans dendritic cells

Figure 9-4 B cell processing antigen
Table 9-1 Summary of Roles of Professional Antigen Presenting Cells

Sentinel dendritic cells are constitutively active and B7 is a co-stimulatory molecule important in activating T cells.

<table>
<thead>
<tr>
<th>Cell</th>
<th>Sentinel Dendritic state</th>
<th>macrophage state</th>
<th>B state</th>
</tr>
</thead>
<tbody>
<tr>
<td>uptake</td>
<td>endo- and phagocytosis</td>
<td>phagocytosis</td>
<td>receptor-mediated endocytosis</td>
</tr>
<tr>
<td>MHC II</td>
<td>constitutive</td>
<td>inducible</td>
<td>constitutive</td>
</tr>
<tr>
<td></td>
<td></td>
<td>induced</td>
<td>higher levels</td>
</tr>
<tr>
<td>B7</td>
<td>constitutive</td>
<td>inducible</td>
<td>inducible</td>
</tr>
<tr>
<td></td>
<td></td>
<td>induced</td>
<td>induced</td>
</tr>
<tr>
<td>activates</td>
<td>naïve, effector and memory T cells</td>
<td>effector and memory T cells</td>
<td>naïve, effector and memory T cells</td>
</tr>
</tbody>
</table>

II. Self-MHC restriction

Figure 9-5
Guinea pig

Figure 9-6
T<sub>H</sub> cell cartoon

Table 9-2- MHC II restriction

<table>
<thead>
<tr>
<th>Self MHC/ self-antigen</th>
<th>Foreign MHC/ foreign antigen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Self MHC/ foreign antigen</td>
<td>Foreign MHC/ self-antigen</td>
</tr>
</tbody>
</table>

A. T<sub>H</sub> cells and class II MHC, Rosenthal and Shevach

1. Used guinea pigs from two different highly inbred strains.

2. Added viral antigen to the macrophages from one strain (strain 2).

3. Gave the macrophages time to process and then isolated them. These are the “antigen-pulsed” macrophages.
4. Exposed Tₜ cells to these macrophages.

5. If the Tₜ cells were from the same strain (2), they were stimulated to divide and differentiate.

6. If the Tₜ cells were from a different strain (13) then they would not respond.

Table 3-3  Experimental Results

<table>
<thead>
<tr>
<th>Tₜ cells from strain 2</th>
<th>Macrophages from strain 2</th>
<th>respond</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tₜ cells from strain 13</td>
<td>Macrophages from strain 2</td>
<td>no response</td>
</tr>
<tr>
<td>Tₜ cells from 2/13 hybrid</td>
<td>Macrophages from strain 2</td>
<td>respond</td>
</tr>
</tbody>
</table>

7. If the Tₜ cells were from an F₁ strain from the cross of 2 and 13, then they would respond. Such cells will share a haplotype with either strain.

8. This showed that Tₜ cells are class II MHC restricted - meaning they will only respond to antigen presented on class II MHC molecules whose genes they themselves possess.

B. Tₐ cells and class I MHC, Zinkernagel and Doherty, used mice.

1. Immunized mice with LCM (lymphocytic choriomeningitis) virus.

2. Extracted cells from the spleen, which included Tₐ cells with receptors specific to virus antigen.

3. They then mixed these cells with LCM virus-infected cells and checked to see if the Tₐ cells could kill the infected cells.
4. If the infected cells shared a haplotype with the Tc cells, then the Tc cells attacked the infected cells.

5. If the infected cells did NOT share a haplotype, then the Tc cells ignored them, even though they could recognize the viral antigen perfectly well.

<table>
<thead>
<tr>
<th>Tc cells from strain A</th>
<th>LCM-infected cells (strain A)</th>
<th>respond</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tc cells from strain B</td>
<td>LCM-infected cells (strain A)</td>
<td>no response</td>
</tr>
<tr>
<td>Tc cells from AB hybrid</td>
<td>LCM-infected cells (strain A)</td>
<td>respond</td>
</tr>
</tbody>
</table>

6. This showed that Tc cells are class I MHC restricted: If the viral antigen was not displayed on a self-class I MHC, then the Tc cells could not respond to it.

C. T-cell Antigens, Townsend *et al.*

1. The Tc cell recognized internal matrix and nucleocapsid proteins better than envelope proteins.

2. Moreover they were recognizing short peptide sequences, not the proteins as a whole.

3. Synthetic peptides with these sequences worked just as well to target flu-infected cells.

III. Cytosolic Pathway: Class I Processing and Presentation

Figure 9-9 CTL recognizing LCM-infected cell

Figure 9-10 Proteasome
A. Peptide Generation by Proteasomes
Zogby http://www.youtube.com/watch?v=p2uC5j0hK0A

1. Protein turnover is a normal feature of cell metabolism.

2. Proteins are targeted for destruction by complexing with another small protein, ubiquitin.

3. Complexing with ubiquitin sends a protein to the interior of a proteasome.

4. LMP2 and LMP7, (coded for by genes embedded in the class II region of the MHC complex) two additional protein subunits, attach to the proteasome and promote the degradation of proteins into peptides of the correct size (9 amino acids) and composition (carboxyl terminal is hydrophobic or basic).
B. Transport to the RER

1. The peptides so generated bind to TAP 1 and 2 proteins (also coded for by genes embedded in the class II region of the MHC complex) embedded in the RER.

2. TAP proteins (transporters associated with antigen processing) have the following structure:
   a. heterodimer
   b. cytosolic binding site for peptides
   c. ATP hydrolysis function near the binding site (variant of ATP-binding cassette proteins)
   d. hydrophobic anchoring regions with three switchbacks per peptide

3. The genes for the TAPs and the LMP2 and 7 all map within the class II (not a typo) region and are polymorphic.

4. The TAP 1 and 2s bind to the peptides, hydrolyze ATP, and drive them into the lumen of the RER.

C. Assembly with and Display by Class I MHC

1. Recall that the class I MHC peptides are synthesized on the RER, and that the \( \alpha \) chain is inserted into the membrane, and the \( \beta \) microglobulin chain sent though to the lumen.

2. Calnexin, a molecular chaperon, associates with the \( \alpha \) chain, promoting its proper folding.

3. The \( \beta \) microglobulin chain can then associate, releasing the calnexin.

4. The complex now associates with the chaperone calreticulin and with tapasin, which brings the TAP transporter over to the complex.
5. ERp57 then binds over the whole distal end of the complex.

6. When the TAP protein hands off the peptide to the cleft, the antigenic peptide stabilizes the MHCI molecule and allows it to exit the RER to the Golgi.

7. The antigenic peptide displaces the ERp57 and the chaperones dissociate from the class I MHC - peptide complex.

8. After sorting in the Golgi, the vesicle with the class I MHC - peptide complex is released by exocytosis and the complex becomes part of the plasma membrane extending to the exterior.
IV. Exogenous Pathway: Class II Processing and Presentation

A. Internalizing Antigen

1. Phagocytosis - engulfment of whole particles with pathogens - macrophages

2. Endocytosis - internalizing membrane carrying whatever the membrane is bound to - B cells internalize antigen as they recycle patches of membrane containing M and D antibodies.

![Figure 9-19 endocytosis](image1)

![Figure 9-20 hydrolysis](image2)

B. Hydrolysis in Vesicles

1. Goes through a series of three vesicle types, each one more acidic and hydrolytic.
   a. early endosome, pH 6.0 - 6.5
   b. late endosomes of endolysosomes, pH 5.0 to 6.0
   c. lysosomes (or phagolysosomes), pH 4.5 - 5.0

2. Lysosomes contain a variety of hydrolytic enzymes that will not only chop up foreign proteins, but it will also remove any carbohydrate and lipids attached to them and hydrolyze them.

3. The net result is a collection of peptides, most of which are 13 to 18 amino acids long, and an array of miscellaneous digestive products.
C. Joining Up with the Class II MHC

1. Class II MHCs are synthesized by the RER, just like class I.

2. However, vesicles with class II MHC are targeted by the Golgi differently from those with class I MHC, thus encountering peptides that not only come from a different source, but which have also been processed somewhat differently.

3. Three pairs of a complete class II MHC $\alpha \beta$ heterodimer associate with a trimer of invariant chains (Ii).

4. The invariant chain plays an important role in the sorting process:
   a. blocks the cleft, preventing premature binding of the wrong (cytosolic) antigen.
   b. acts as a chaperone, to help fold the $\alpha \beta$ peptides into proper conformation
   c. helps them exit the RER.
   d. tags them for recognition by the Golgi in the sorting to fusion with endocytic vesicles.

5. Class II MHC-invariant complexes leave the Golgi and fuse with the early endosomes.

6. As the antigen and complexes move from the early to the late endosomes to the lysosomes, the invariant chains get degraded, leaving behind a fragment, called the CLIP, which still blocks the cleft.
7. At the end of hydrolysis, a non-classical MHC (HLA-DM) removes the CLIP, exposing the class II MHC to the antigenic peptides.

8. The binding of antigen stabilizes the class II MHC complex, allowing it to be transported to the surface and displayed.

![MHC II binding site, with antigen](image)

Figure 9-22, MHC II binding site, with antigen

V. Making Sense of Variations

A. Cross presentation

1. APCs pick up exogenous antigen and put it on MHCI to activate Tc cells.
2. Endosomes with peptides digested from a pathogen travel to RER and fuse.
3. Antigen in the lumen can now attach to MHCI

![Cross presentation, Shown in context with traditional presentation on MHC I and II](image)

Figure 9-23 Cross presentation, Shown in context with traditional presentation on MHC I and II
Figure 9-24, CD1 model compared with MHC I and II

Figure 9-25 Lipid presentation, compared with presentation on MHC II
B. Non-Peptides

1. T cells can also recognize bacterial lipid derivatives. These are presented by a group of non-classical MHCs, the CD1 family of 5 genes (A through E), discussed previously.

2. The overall **structure** of the CD1 molecules resembles that of MHC I. Both are peptides made up of 3 domains, binding antigen between the α1 and a2, and associating with β microglobulin.

3. However, the lipid antigens that fit on these molecules must generated by hydrolysis in an endosome (phagolysosome) and then presented to TH cells. In this respect the CD1s **function** more like a class II MHC.

4. However the pathway is quite strange. The CD1 heads to the cell surface, backs up into the lysosomes, picks up the antigen and the heads back to the surface.

5. T cells seem to receive these rather differently as well. They may use a somewhat different receptor, with the γδ receptor, and no CD4 or CD8. T cells with αβ receptors have been shown to bind these as well, using CD4

6. NK cells may also recognize members of the CD1 family displaying autologous (non-bacterial) antigen, and participate in recycling damaged cells identified by these signals.

7. iNKT cells plus CD4 also recognize lipids on CD1

C. Compare and Contrast

1. Compare and contrast the structure and function of Class I and Class II MHC molecules with respect to:
   a. **structure** (Indicate homologies between domains in the two classes and distribution of membrane-spanning regions.)
   b. **location and structure of antigen binding site**
   c. **size of antigen peptide bound and conformation in the binding site**
   d. **intracellular location of synthesis of the MHC and where in the cell the antigen is loaded onto the MHC**
   e. **which cells synthesize either class**
   f. **what type of T cell that they present/display to, the co-receptor they associate with and interaction with the T cell that they promote**
Follow up: The α chains of class I and class II MHC molecules are similar in that they BOTH ______.
A. are composed of two domains
B. form the complete structure of the binding site
C. are anchored to the cell by membrane-spanning domains
D. are unimportant in the recognition process leading to transplant rejection
E. are coded for by genes rearranged in the bone marrow

2. Compare and contrast the processing and presentation of exogenous and endogenous antigen with respect to:
   a. biological source of the antigen
   b. class of MHC molecule that will present it
   c. class of T cell that will respond to the antigen-MHC complex
   d. sequence of events bringing the antigen from wherever it starts out to the MHC molecule.
   e. The accessory proteins used in processing antigen or chaperoning the complex
   f. process leading to display on the cell surface

Follow up: Processing and presentation of antigen for presentation on class I MHC differs from the corresponding process involving class II MHC in that only in the processing for class I ______.
A. can the resulting complex display lipids as well as proteins
B. does the MHC enter the Golgi body with the antigen in the binding cleft
C. will the resulting complex be recognized by both αβ and γδ TCRs
D. is the complex displayed after a vesicle fuses with the plasma membrane
E. requires that a protein be hydrolyzed into a peptide

3. Compare and contrast antigen presentation on the non-classical MHC presenting molecule, CD1 with cross presentation on MHCI. Include:
   a. A comparison of the structure of CD1 and MHC1
   b. Where the molecules are synthesized and ultimately displayed.
   c. The source and chemical nature of the antigen.
   d. Where the antigen is loaded onto the MHC and subsequently displayed.
   e. The T cells binding to the antigen and co-receptors involved.

Follow up: Presentation of antigen on CD1 differs from cross presentation on MHC I in that only the CD1 ______.
A. binds the antigen at a binding site comprised of the junction of two distinct peptides
B. binds peptides of 11 to 12 amino acids
C. presents to T_\text{H} cell
D. displays an antigen derived from exogenous sources
E. is a membrane-bound protein composed of three domain
## Summary Table

Excellent source of double dichotomous choice (MCAT-style) factoid questions

<table>
<thead>
<tr>
<th></th>
<th>Class II MHC</th>
<th>Class I MHC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Function</strong></td>
<td>external antigen presentation</td>
<td>self antigen display</td>
</tr>
<tr>
<td><strong>Cells presenting/displaying</strong></td>
<td>Sentinel dendritic, macrophages, B cells</td>
<td>All nucleated cells except sperm</td>
</tr>
<tr>
<td><strong>Molecule structure</strong></td>
<td>α and β peptides, two domains each</td>
<td>α peptide with 3 domains, associated β microglobulin with 1 domain.</td>
</tr>
<tr>
<td><strong>Source of antigen</strong></td>
<td>phagocytosis</td>
<td>synthesized in cell</td>
</tr>
<tr>
<td><strong>Antigen hydrolyzed in</strong></td>
<td>phagolysosome</td>
<td>proteasome</td>
</tr>
<tr>
<td><strong>Typical length of peptide</strong></td>
<td>13 to 18 amino acids</td>
<td>8 to10 amino acids</td>
</tr>
<tr>
<td><strong>Antigen loaded onto MHC in</strong></td>
<td>phagolysosome</td>
<td>rough ER</td>
</tr>
<tr>
<td><strong>Antigen binding site</strong></td>
<td>open-ended cleft between α and β peptides</td>
<td>closed slot at junction of α1 and α2 peptide domains</td>
</tr>
<tr>
<td><strong>antigen conformation</strong></td>
<td>lies flat</td>
<td>bows outward</td>
</tr>
<tr>
<td><strong>Responding cell</strong></td>
<td>T_H (Helper)</td>
<td>T_C (Cytotoxic)</td>
</tr>
<tr>
<td><strong>TCR binding to antigen</strong></td>
<td>αβ TCR</td>
<td>αβ TCR</td>
</tr>
<tr>
<td><strong>Co-receptor</strong></td>
<td>CD 4</td>
<td>CD 8</td>
</tr>
<tr>
<td><strong>Response</strong></td>
<td>Coordinates immune response</td>
<td>Attacks and kills cell</td>
</tr>
</tbody>
</table>

### References:


MHC polymorphisms: [http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3235490/?tool=pmcentrez](http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3235490/?tool=pmcentrez)


Zinkernagel and Doherty: [https://en.wikipedia.org/wiki/Peter_C._Doherty](https://en.wikipedia.org/wiki/Peter_C._Doherty)
Lecture 10
T-Cell Receptors

“We should never have sought either solace or moral instruction in Nature.” – Stephen Jay Gould

I. Early Work -

A. Learning (or Not) from the Immunoglobulin Receptor

B. Cloning the T-cell receptor gene (Hedrick and Davis)

1. Isolated mRNA from membrane-bound polysomes, on the assumption that the TCR entered the endoplasmic reticulum and inserted itself into the ER membrane by the standard mechanism.

This eliminated 97% of the mRNA, presumably the mRNA on cytoplasmic ribosomes coding for cytosolic, nuclear and mitochondrial proteins. The remaining 3% of messages should code for membrane-bound and secreted proteins.
2. Copied the polysomal (3%) messages using $^{32}$P labeled nucleotide precursors. They now had a radioactive probe to find similar messages in B cells.

3. Used *subtraction hybridization* to get rid of mRNA that wasn’t coding for the receptor. That is, they isolated membrane-bound polysomes from B cells. This fraction should have mRNA for proteins common to both types of lymphocytes, but not those for the TCR. They could then use a large excess of the B-cell mRNA to hybridize with and remove any messages the two cells had in common. Thus most of the radioactive DNA was flushed out by hybridization with the B cell membrane messages.

4. Only 10 different cDNA clones remained, presumably of messages unique to T cells, and including the messages for the TCR. You would expect messages for the $\alpha$ and $\beta$ peptides, the $\delta$, $\varepsilon$, $\gamma$, $\zeta$ and $\eta$ peptides of the CD3, and the CD4 and CD8.

Figure 10-3 Constructing the cDNA probe and isolating the T-cell specific messages via subtraction hybridization
Figure 10-4, identifying the messages that had been transcribed from rearranged genes

5. Checked to see which of these sequences bound to genes in T cells that had been rearranged. The sequences would bind only to the DNA present after rearrangement, and the cDNA would not be able to recognize unarranged gene from macrophages or B cells.

6. Looked at 6 different T-cell lineages, each with a somewhat different version of the gene. Presumably from the receptor mRNA would be different in different lines of cells.
II. T-Cell Receptors: Structure and Roles

A. $\alpha\beta$ versus $\gamma\delta$ receptors

1. The receptor as a heterodimer, made up of either $\alpha\beta$ or $\gamma\delta$ peptides.

2. A T cell can make one kind or the other. Moreover there’s no such thing as a hybrid ($\alpha\delta$ or $\gamma\beta$) receptor.

3. Most T cells make $\alpha\beta$ receptors.

B. Common Elements

1. The structure of both types resembles that of a truncated immunoglobulin.

2. Each chain has a conserved region including a constant “bread and butter sandwich” region typical of the immunoglobulin superfamily.

3. Each chain has a variable “bread-and-butter sandwich” region at the amino terminus with three hypervariable regions in each chain.

4. The transmembrane chains (also conserved and ending at the carboxyl terminal) are unusual in that they have positively charged (hydrophilic) residues that attract other (negatively charged) $R$ groups on the transmembrane signal transducer, CD3.

5. The membrane signaling complex is the same for both receptors.

C. Functional Differences.

1. $\alpha\beta$ T cells must undergo selection to produce a highly specific recognition molecule, each cell having only one kind and with a huge variety of different possible kinds of these receptors produced by different cell lineages.

2. $\gamma\delta$ T-cell receptors also undergo selection to produce a specific receptor, but from a defined repertory of receptor types evolved in response to pathogens most often encountered by that organism.
3. γδ T cells have receptors that target are relatively limited and defined range of antigen. For example, most human γδ T cells recognize lipid antigen characteristic of tuberculosis bacteria and parasites (malaria and leishmaniasis).

4. γδ T cells function very differently from αβ T cells:
   a. can react with antigen directly, that is, not presented on MHC
   b. can also recognize lipid antigens presented on non-classical MHC
   c. can bind to non-classical MHC (T22), which does not bind antigen and seems to up-regulate their activity.
   d. typically has neither CD4 nor CD8.
   e. can both signal and attack (attack resembles that of Tc and NK cells, inducing apoptosis)
   f. can phagocytize and present antigen (Brandes, et al, 2005).

5. γδ T cells are important in patrolling mucosa and epithelia and in wound healing.

6. Disruptions in γδ T-cell regulation are associated with autoimmune disorders.

7. From now on, unless otherwise specified, when I talk about a T cell receptor, I mean an αβ.

Table 10-1 the αβ and γδ Receptors Compared

<table>
<thead>
<tr>
<th>receptor</th>
<th>αβ</th>
<th>γδ</th>
</tr>
</thead>
<tbody>
<tr>
<td>structure</td>
<td>two peptides, + charged anchors, 147° bend</td>
<td>two peptides, + charged anchors 111° bend</td>
</tr>
<tr>
<td>diversity</td>
<td>T_h and T_c: highly</td>
<td>limited (quasi innate)</td>
</tr>
<tr>
<td>requires MHC</td>
<td>yes (MHC I, II and CD1)</td>
<td>no, but may use CD1 or T22</td>
</tr>
<tr>
<td>requires co-receptors</td>
<td>CD4 or CD8</td>
<td>not usually, but may have Fc and Toll-like receptors</td>
</tr>
<tr>
<td>phagocytosis and antigen presentation</td>
<td>no</td>
<td>yes</td>
</tr>
<tr>
<td>location</td>
<td>mucosa</td>
<td></td>
</tr>
</tbody>
</table>
III. T-Cell Receptor Genes

Figure 10-6, Location of TCR genes

A. Gene Location (Humans)

1. The α gene family is located on chromosome 14, long arm
2. The β gene family is located on chromosome 7, long arm
3. The γ gene family is located on chromosome 7, short arm
4. The δ gene family is also located on chromosome 14, very strangely in the middle of the α gene family, between the V_α and J_α genes.

B. Gene Regions (Humans)

1. Compare with Ig genes: the set-up resembles that of the immunoglobulin genes, with the α and γ equivalent to the light and the β and δ equivalent to the heavy.

2. Numbers of Gene Regions: Please note that this table refers to humans. Use it as an approximation.

<table>
<thead>
<tr>
<th>Gene\Gene region</th>
<th>V</th>
<th>D</th>
<th>J</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>α gene</td>
<td>~40</td>
<td>-</td>
<td>~50</td>
<td>1</td>
</tr>
<tr>
<td>β gene</td>
<td>~40</td>
<td>2 (functional)</td>
<td>6 and 7</td>
<td>2</td>
</tr>
<tr>
<td>γ gene</td>
<td>~5 (functional)</td>
<td>-</td>
<td>~ 5</td>
<td>2</td>
</tr>
<tr>
<td>δ gene</td>
<td>~3 (or more)</td>
<td>~ 3</td>
<td>~ 4</td>
<td>1</td>
</tr>
</tbody>
</table>
C. Mechanisms of Gene Rearrangements: very much like those that go on in immunoglobulin genes. Feel free to review the Lecture 5 videos.

![Diagram of RSSs (Recombination Signal Sequences) of TCR genes]

**Figure 10-7** the RSSs (Recombination Signal Sequences) of TCR genes

1. The Recombination Signal Sequences are the same.
   a. Have a heptamer (7 base) palindrome next to the region to recombine (downstream on V, upstream on J and on both sides of the D).
   b. Next to the palindrome is either a one or a two-turn (12 or 23 base) sequence.
   c. Next to the turn sequence is the AT-rich nonamer, or nine base sequence.

2. The RAG1 and RAG2 enzymes are the same one used in B cells.

3. A one-turn sequence must join to a two-turn sequence.

4. The joint between two regions will have:
   a. a variable number of palindromic bases
   b. bases added through P-nucleotide addition
   c. bases added through N-nucleotide addition (this includes both β and δ and α and γ genes.)
   d. none of the bases from either the turn sequences or the AAT rich nonamers

D. Gene Expression:

1. similar to the process in Ig genes

2. All messages have membrane-spanning exons.
3. All four possible peptides extend into the lumen of the ER, anchored into the membrane.

4. Vesicles with receptor fuse with the plasma membrane.

5. More details in animation in the next clip.

IV. Gene Rearrangement in Action

Figure 10-8  TCR Genes, showing location of functional regions

A. Alpha Rearrangement

1. removes the δ gene.

2. leaves variable number of VL regions in the gene.

3. only activates the promoter of the αVL joined to the J

4. leaves variable numbers of Js.

5. leaves intron preceding the C.
B. Beta Rearrangement

1. leaves variable number of VL regions in the gene.

2. only activates the promoter of the βVL joined to the D (or the J if the D gets left out)
Figure 10-12 results of this rearrangement

Figure 10-13 shows a different rearrangement,

Figure 10-14 shows results of rearrangement shown in figure 10-14 (note absence of D and presence of remaining downstream sequences.)

3. leaves variable numbers of Js
4. leaves intron preceding the C
5. In this you use the first C1, will leave the J2s and C2 as well.
C. Delta Rearrangement.

1. leaves both the whole α gene and variable number of VL regions in the δ gene.
2. only activates the promoter of the δVL joined to the D (or J).
3. may add more than one D

Figure 10-16 Delta gene rearrangement incorporating two D regions.

4. leaves variable numbers of Js
5. leaves intron preceding the C

D. Gamma Rearrangement

1. leaves variable number of VL regions in the gene.
2. only activates the promoter of the γ VL joined to the J.
3. leaves variable numbers of Js.
4. leaves intron preceding the C
E. Subsequent Expression: Common Elements:

1. The region is made up of 4 exons, each corresponding to a functional domain.
   a. Constant Ig domain (β pleated sheet switchback)
   b. Extracellular connecting sequence
   c. Transmembrane region
   d. Cytoplasmic anchoring tail

2. Transcription begins at the promoter (activated by splicing) and transcribes through whatever constant region it reaches first and then stops.

3. The message precursor is capped and gets a poly A tail.

4. Processing removes any extra Js.

5. Translation of the leader attaches the ribosome to the ER.

6. All four TCR peptides will insert into the lumen
   a. The C terminal remains anchored to the ER membrane
   b. The leader is clipped off.
   c. Enzymes in the Golgi oxidize the SH groups to form disulfide bonds
   d. Vesicles with receptor fuses with the plasma membrane.
V. Diversity and its Constraints

A. Sources

1. Combinatorial joining - The random joining of V and J segments is still a geometric generator of diversity.

   a. \( \alpha \) and \( \gamma \) have the following segments: multiple leaders plus Vs, multiple Js, and single C (or constant).

   b. \( \beta \) has multiple leaders plus Vs, and two repeats of a single J, D and C.

   c. \( \delta \) multiple leaders plus Vs, (because it's in the middle of \( \alpha \), it sometimes exchanges Vs with it), multiple Ds and Js, and a single C

2. Alternative joining - sometime the \( \beta \) chain leaves out the D segment altogether. The \( \delta \) can even add in more than one D segment.

3. Junctional flexibility - variation in exactly where the segments are joined.

4. P and N nucleotide addition occurs in all chain rearrangements, whereas for immunoglobulins, only the heavies do N.

B. Constraints

1. A functioning TCR must recognize an MHC, either I or II.

2. It is thought that the V regions accommodate this.

3. However, when it comes to recognizing antigen, the TCR must be prepared for extreme diversity. This seems related specifically to one of the recognition regions, CDR3.

4. On the other hand, one thing that the genes do not do is undergo affinity maturation: no somatic mutation followed by selection.
VI. The TCR Receptor Complex:

A. CD3, the receptor complex

1. complex of three dimers associated with the $\alpha$ and $\beta$ chains (or $\gamma$ and $\delta$)
   
   a. $\gamma \varepsilon$ heterodimer
   b. $\delta \varepsilon$ heterodimer
   c. $\zeta \zeta$ homodimer or $\zeta \eta$ (zeta-eta) heterodimer (only 10%)

2. $\zeta$ and $\eta$ are splicing isoforms: coded for by the same gene.

3. peptide structure
   
   a. $\gamma, \delta,$ and $\varepsilon$ chains are all part of the immunoglobulin superfamily.
   
   b. $\zeta$ and $\eta$ are not, having only 9 amino acids to the exterior and a long extended cytoplasmic tail.
   
   c. All CD3 peptides have a negatively charged aspartic acid in the transmembrane regions which causes them to salt bridge with a positively charged R groups in the transmembrane domain to the TCR.
   
   d. All peptides have an ITAM (immunoreceptor tyrosine-based activation motif) at the interior, the $\zeta$ and $\eta$ cytoplasmic tail having three each.
   
   e. The ITAMs function like those in the B cell receptor: they attract other tyrosine kinases and do not autocatalyze the transfer of phosphates themselves.

B. CD4

1. used by $T_H$ cells to bind class II MHC

2. immunoglobulin superfamily protein

3. functions as monomer
4. 4 extracellular immunoglobulin domains

5. N-terminal domain binds to the base of MHC II in a hydrophobic pocket for by the junction of the α2 and β2 domains.

6. transmembrane region

7. C-terminal cytoplasmic tail with 3 serines that can be phosphorylated
C. CD 8

1. used by $T_C$ cells to bind class I MHC
2. immunoglobulin superfamily protein
3. functions as $\alpha-\beta$ heterodimer or $\alpha-\alpha$ homodimer (link by disulfide)
4. extends out from the membrane as a stalk ending one Ig domain per peptide at the N-terminal end.
5. double Ig domains wrap around the $\alpha_3$ and also touch $\alpha_2$ domains and the $\beta$ macroglobulin.
6. transmembrane region
7. cytoplasmic tail with several amino acids that can be phosphorylated

VII. Alloreactivity: Once of Life’s Great Mysteries

A. The Problem

1. Tissue transplants are rejected if the transplant has MHC molecules not found in the host, which is almost always.
2. $T_H$ cells recognize foreign class II, and $T_C$ cells foreign class I.
3. The tissue is destroyed by $T_C$ cells.
4. But, given what we’ve studied up to now, they shouldn’t be able to do that. The TCRs seem to be recognizing the foreign MHC directly and then mounting an immune attack.

B. Evidence and Speculation

1. 1 - 5% of all T cells are alloreactive - that is, they can bind non-self MHCs. This is a lot more than can recognize any one native MHC-antigen complex.
2. There is some evidence that T cell recognize foreign MHC via a binding mechanism somewhat different from that used to detect antigen attached to self MHC.
3. The problem may in part arise inability of the T-regulatory cells to recognize a transplant and then protect it from the random attack of NK, Tc or γδ T cells.

4. Moreover, most authors ignore the possible role of a γδ T cell initiating the response. Which is odd, because we know they function in autoimmunity, do not to require MHC for recognition, and both attack and signal other cells to attack. On the other hand, they’re supposed to recognize primarily lipids and we know little about the selection process (if any) that precedes their release form the thymus.

What’s Where (Human):

- Chromosome 1 - CD1 family of non-classical MHC molecules, CD3 zeta/eta
- Chromosome 2 - κ light chain immunoglobulin, CD8
- Chromosome 4 - J chain
- Chromosome 6 - MHC complex
- Chromosome 7 - γ and β TCR chains
- Chromosome 11 - CD3 gamma, delta and epsilon
- Chromosome 12 - CD4
- Chromosome 14 - heavy chain of immunoglobulin α and δ TCR chains
- Chromosome 15 - β microglobulin of class I MHC
- Chromosome 17 – Ig beta (CD79b)
- Chromosome 19 – Ig alpha (CD79a)
- Chromosome 22 - λ light chain immunoglobulin

This is just for fun. After teaching immunology for several years, I began to wonder if there was any relationship between where gene were located and the location of other genes that they might need to coordinate with. So far, I don’t have a clue.

References:


Brandes et al, Science Express (sciencemag.org/cgi/content/abstract/1110267v1) on γδ T cells as antigen presenters.

Hedrick and Davis experiment (the fun historical narrative): http://stanmed.stanford.edu/2014fall/swashbuckler.html

Lecture 11

T-cell Development

“For a successful technology, reality must take precedence over public relations, for nature cannot be fooled.” - Richard Feynman

I. Maturation in the Thymus

A. Early Events

1. Hematopoiesis

2. Some stem cells differentiate into lymphoid precursors – *ikaros*, a Zn finger protein transcription factor.

   ![Figure 11-1 Zinc-finger protein](image1)
   ![Figure 11-2 Delta-notch interaction](image2)

   3. Signals from surrounding cells to notch activate lymphoid precursors to differentiate into T-cell precursor (or T-cell progenitors or thymocytes).

   4. These cells change their surface protein expression and migrate to the thymus.

   ![Figure 11-3 thymus](image3)
B. Double Negative Transitions (division somewhat arbitrary, think stages of mitosis)

1. DN 1 (first double negative) T-cell progenitors (thymocytes or pro-T cells) analogous to that of a pro-B cell (progenitor B cell).
   a. Cells migrate to thymus, no TCR (receptor)
   b. neither CD4 nor CD8 (CD4- CD8- or double negative) NOR initially CD25, which is one of three peptides of the IL-2 receptor. Much more later, as this is fundamental in stimulating immune cell proliferation and differentiation.
   c. genes for TCR have not rearranged
   d. express membrane-bound receptor, c-Kit (CD117), which receives a paracrine factor call alternatively stem cell factor or steel. The signal tends to keep a cell in an undifferentiated state.
   e. membrane-bound CD44 (an adhesion molecule that come in several forms. Some target to bone marrow, but this version seems to target to thymus)

2. DN2- The T-cell progenitors stop dividing and begin rearranging their genes.
   a. Cells remain in cortex just to the interior of the capsule
   b. They begin reducing expression of c-Kit, reduce CD44 expression, express CD-25, allowing them to assemble the complete IL-2 receptor.
   c. Turn on RAG genes, the same genes used to cut and paste Ig genes.
   d. Turn on CD3 co-receptors.
   e. A subpopulation of cells rearranges the \( \gamma \delta \) genes. Most frequent during gestation and declines to about 5% in adulthood. These cells exit the thymus and head to epithelia.
   f. Most thymocytes begin to rearrange the \( \beta \) gene (pro-T cell).

3. DN3
   a. cells still in sub-capsular cortex
   b. c-kit or CD44 and CD 25 still present, but declining
c. \( \beta \) chains produced by productive rearrangement combine with preT\( \alpha \) chains (analogous to surrogate light chains in B cells) and CD3 signaling peptides (loosely analogous to the B-cell \( \alpha \) \( \beta \) dimer) to form a pre-T-cell receptor (pre-TCR of pre T cell).

4. DN4
   a. Cell penetrate to cortex (which is still the outer layer).
   b. \( \beta \) gene undergoes allelic exclusion, allowing rearrangement of the \( \alpha \) gene, although this is at first held in check.
   c. Induces expression of BOTH CD4 and CD8, leading to the double positive state.

<table>
<thead>
<tr>
<th>Double Negative to Double Positive Development: Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DN1</strong></td>
</tr>
<tr>
<td>DN2</td>
</tr>
<tr>
<td>DN3</td>
</tr>
<tr>
<td>DN4</td>
</tr>
<tr>
<td>DP</td>
</tr>
</tbody>
</table>

Figure 11-4 CD3 co-signaling peptides  Figure 11-5 DN3 receptor with Pre-alpha
II. Double Positive (DP) Events

A. Completing the Receptor

1. RAG-2 first rises

2. Thymocytes begin to rearrange the α gene and then express it.

3. RAG-2 declines

4. Expanded clones with the same β gene have many cells, each of which can rearrange to a different α gene, increasing diversity.

5. Cells making good αβ TCR and CD3 have a functioning receptor and survive

B. Displaying Co-Receptors

1. Cell no longer express any c-kit, CD44 or CD25, although they will express the two other subunits of the IL-2 receptor.

2. Cells now express both CD 4 AND CD8 (double positive).

C. Beginning Selection – the process demands that

1. Cells must decide which MHC to respond to, and thus whether they should continue to make CD4 or CD 8.

   a. Cells that can bind MHCI will keep the CD8 and lose the CD4.

   b. Cells that kind bind MHCII will keep the CD4 and lose the CD8.

2. Cell must not recognize self-antigen.
a. Vast possible number of αβ TCRs ($10^{15}$ – a number so large as to be meaningless)

b. The receptors can theoretically attach to almost anything.

III. Selection: Fully 98% of all thymocytes that start out in the thymus die there.

A. Positive Selection: results in significantly more cell deaths than does negative

1. Immature thymocytes in cortex of the thymus contact stromal cells (epithelial, macrophages, and dendritic cells.)

2. Stromal cells express MHC, both class I (as will any cell) and class II.

3. Thymocyte TCR binding to MHC molecules, signals inhibition of apoptosis.

4. Only developing T cells that can bind to and recognize self-MHC survive.

5. T cells become single-positive, expressing CD8 or CD4 depending on which class of MHC they happen to recognize.

   a. Those recognizing class I continue to express CD8.

   b. Those recognizing class II continue to express CD4.

   c. If you knock out the gene for MHC II, the mice will not produce CD4+ cells.

   d. If you knock out the gene for MHC I, the mice will not produce CD8+ cells.

6. Thus cells with no affinity for MHC are deleted. Positive means that there must be some affinity or the cells won’t survive.
B. Negative Selection, against those cells that bind MHC plus self-antigen, which results in self-tolerance.

1. Takes place in medulla

2. Stromal cells of the medulla produce a varied buffet of self-antigens, including many proteins normally characteristic of differentiated tissues.

3. They do this by expressing an unusual transcription factor, AIRE.
   a. the Swiss army knife of transcription factors, having two plant Hodo Zn fingers, 4 nuclear receptor motifs, a SAND domain and N terminal similar to SP100.
   b. randomly up-regulates transcription in thymic epithelial and dendritic cells, which present antigen. This exposes developing thymocytes to many more of the body’s antigens than developing B cells encounter in the bone marrow.
   c. Mutations in the aire (autoimmune regulator) gene are inherited in humans as a classical Mendelian autosomal recessive, producing a type of multi-organ autoimmune disorder call APECED.

4. The selection process removes:
   a. cells with a very high affinity for MHC all by itself.
   b. cells with affinity for MHC plus self-antigen.

5. The process leaves alive only those T cells that respond to altered-self: MHCs with non-self-antigens.

6. Cell that survive all this exit into the circulation

C. Issues in Selection, the Goldilocks Principle

1. How then could not binding to self-MHC lead to apoptosis at the same time that binding to it too avidly (whether or not it has antigen on it) will also lead to apoptosis?

2. Something allows just those cells with binding specificity for self-MHC displaying non-self-antigen to survive the process. How?

3. It a cell can bind to MHC weakly, using the 1 and 2 loops of the receptor, and if it encounters an antigen outside the thymus, that antigen will be foreign and will cause the whole complex to bind tightly enough to kick off a response.
4. The strength of the binding (avidity) between the TCR complex and the MHC-antigen complex of the presenting cell determines whether the T cell will live or apoptose.

a. If binding between receptor and MHC too weak, the T cell dies. It can’t recognize the antigen on the MHC.
b. If binding between receptor and MHC is too strong, the T cell dies. It will react whether there is an antigen present or not, or will react to self-antigen plus MHC.
c. Only a medium binding strength permits survival, because this means a cells is primed to bind an antigen it has yet to encounter, presumable a foreign one.

IV. CD4+ T<sub>H</sub> Cells: Types and Functions
Recall that T<sub>H</sub> activation is the necessary and central event for humoral response (production of antibodies by B cells) and cell-mediated (T<sub>C</sub>) responses.

A. Determination of Subclass

1. T<sub>H</sub>1
2. T<sub>H</sub>2
3. T<sub>reg</sub>
4. T<sub>H</sub>17 (T<sub>H</sub>3) - extra-cellular bacteria, fungi and parasites
   a. Reciprocally regulated with T<sub>reg</sub>. IL6 diverts T<sub>reg</sub> to highly proinflammatory T17 IL 25 and retinoic acid negatively, diverts to T<sub>reg</sub>.
   b. Involved in decision as to whether or not to produce an immune response or ignore the antigen.
   c. Gamma delta cells and some sentinel dendritic cells also secrete IL-17.
   d. Improper up-regulation of T<sub>H</sub>17 also involved in autoimmune diseases.
   e. IL-17 (not just from T<sub>H</sub> cells), is involved in barrier integrity.
   f. Production triggered by IL-23, versus IL-12 for T<sub>H</sub>1

![Figure 11-10, T<sub>H</sub> cell determination](image)
B. Overview of the Process

1. APC presents an antigenic peptide on a class II MHC molecule.

2. T\textsubscript{H} cell binds this complex with a TCR - CD3 receptor, aided by CD4.

3. A cascade of signals, beginning at the cell surface and entering the nucleus, pushes the T\textsubscript{H} cell into a cell division cycle (out of G\textsubscript{0}).

4. This signal process necessarily involves transcriptional activation
   a. immediate genes (within a half hour) - genes for transcription factors
   b. early genes (1 - 2 hours) include interleukins and their receptors.
   c. late genes - more than 2 days after stimulus - cell adhesion molecules.

Figure 11-12 Cascade Summary
C. Superantigens - Not a Good Deal

1. Superantigens are viral or bacterial proteins that bind to ternary TCR-antigen-MHC complex in such a way as to nail them together.

2. This excessively strong binding results in hyperstimulation of the T<sub>H</sub> cell.

3. Include exotoxins secreted by gram-positive bacteria, including those that cause:
   a. staphylococcal enterotoxin
   b. toxic shock toxin
   c. exfoliative dermatitis

4. Binding is non-specific for antigen, and so different populations of T<sub>H</sub> cells are stimulated.

5. The overproduction of cytokines can kill you.

Figure 11-13 Superantigen  Figure 11-14 TCR after antigen binding

Figure 11-15, Lipid raft, start of activation
V. **T**<sub>H</sub> **C**ell Activation Pathway - forming the immunological synapse. Specific TCR-coupled Pathways

A. Signal binds to receptor.

1. TCR binds to MHC II plus antigen
   a. $\alpha\beta$ heterodimer forming the ligand (MHC-antigen complex) binding unit.
   b. CD3 complex - $\gamma\epsilon$ and $\delta\epsilon$ peptides
   c. Homodimer of $\zeta$ (zeta) chains

2. CD-4 assists binding, locking on to constant domain (carboxyl end) of the exposed MHC II.

![Figure 11-16 Synapse- mid-formation](image1)

3. TCR complexes cluster with one another.
4. CAMs for ring surrounding the cluster.

![Figure 11-17 Synapse, mature](image2)
B. Membrane signaling complex assembles.
   1. TCR/CD3 initially excluded from "lipid rafts," regions of the plasma membrane rich in sphingomyelin, glycosphingolipids and cholesterol.
   2. Lipid rafts have \( p56^{Lck} \), associated with CD4 or CD8 co-receptors.
   3. Binding of antigen to TCR causes co-receptor to associate, drawing CD3 complex into the lipid raft.
   4. Cell adhesion molecules associate (integrins bound to IgCAMs) - CD45 to CD22 and reorganize into "bulls-eye."

C. Second messenger(s) generated.
   1. \( p56^{Lck} \) phosphorylates the ITAMs, providing a docking site for ZAP 70.
   2. ZAP-70 (zeta associated protein) gets phosphorylated by \( p56^{Lck} \) and activated.
   3. This kicks off a variety of pathways.

D. Proteins activity regulated by the addition (kinase) or removal (phosphatase) of phosphates.
   1. ZAP-70 phosphorylates a variety of molecules.
   2. Recruits mediators to complex.
   3. Activates phospholipase C.
   4. Activates a G protein

E. Signals amplified by enzymes cascades.
   1. DAG-IP3 via hydrolysis of membrane phospholipid by phospholipase C, causes ER to release \( \text{Ca}^{2+} \).
   2. \( \text{Ca}^{2+} \) release activates NFAT (T-cell specific transcription factor) via pathways involving calmodulin and calneurin.
   3. DAG activates the transcription factor NF-\( \kappa \)B.
   4. Activation of Ras, the G protein.
F. Transcription factors alter genes expression.

1. NFAT and NF-κB, which turn on cytokine genes, including IL-2: up-regulates the innate inflammatory response as well.

2. Transcription factors activated by MAP kinases turn on genes that trigger cell division.

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G. Process requires co-stimulation

1. Signal 1 - Binding of antigen to the CD3 complex is necessary, but not sufficient,

2. Signal 2 – A non-specific co signal is also required.

3. Signal 2 comes mostly from the B7 on the antigen presenting cell binding to the CD28 on the T cell. CD28-B7 co-stimulation initiates a primary response.

4. Within 48 hours the cell will leave G0, enlarge, and begin cell division, begin transcribing the message for both IL2 and the α chain of high affinity version of its receptor and enter into a positive feedback loop.

5. However, binding of B7 to CTLA-4, a similar immunoglobulin superfamily protein, will **downregulate** T-cell development. Two slightly different forms of
B7 are specific for each molecule,

6. Resting T-cells initially have no CTLA-4, but begin to produce it during activation.

7. Clonal anergy: If the APC doesn’t have B7 to bind to CD28, it turns it off.

Figure 11-20  B7 / CTLA4

Before you go – don’t skip the roll playing video, in which a Rice class illustrates the movement of T-cell Receptors into the immune synapse.

References:


On Th17; [http://ndt.oxfordjournals.org/cgi/content/full/23/3/816](http://ndt.oxfordjournals.org/cgi/content/full/23/3/816)
Lecture 12
Cytokines and signaling

“If all the matter in the universe except the nematodes were swept away, our world would still be dimly recognizable, and if, as disembodied spirits, we could then investigate it, we should find its mountains, hills, vales, rivers, lakes and oceans represented by a thin film of nematodes. The location of towns would be decipherable, since for every massing of human beings there would be a corresponding massing of certain nematodes. Trees would still stand in ghostly rows representing our streets and highways. The location of various plants and animals would still be decipherable, and, had we sufficient knowledge, in many cases even their species could be determined by their erstwhile nematode parasites.”

I. Information Transfer

A. Cell to Cell Contact
   1. T<sub>H</sub> cells are activated by APCs, using antigen presented on MHC II.
   2. B cells, once activated by antigen, contact T cells (the “immune synapse”) for information exchange.
   3. Direct contact with infected or malignant cells activates T<sub>C</sub> cells.
   4. Pathogens contacting macrophages trigger an inflammatory response via toll-like receptors.

B. Ways to transfer information
   1. juxtacrine- touching
   2. paracrine- local
   3. hormone- at a distance, via circulation

C. Paracrine Signaling: naming conventions
   1. Cytokines (refers to cell to cell) or Interleukins (IL – refers to white cell to white cell)
      a. secreted from one leukocyte and act on another, although they often affect other cells as well.
      b. numbered 1 through ?
c. Some cytokines (especially those identified early on before scientists developed a theoretical framework to discuss their function) have common names, for example:

i. Interferon (IFN) Interferon is a type of interleukin, and more generally, a type of cytokine

ii. tumor necrosis factor (TNF) – identified because it helped in getting rid of malignant cells. Turns out to be a powerful, general immune up-regulator

2. Chemokines

a. Signal movement of cells

b. Attach to seven-span receptors

c. Activate G-proteins

II. Cytokine Receptors and Ligands

Figure 12-1 Cytokine Receptors
A. Tumor Necrosis Factor Receptor (TNFα is one ligand.)

1. Receptor (TNFR or CD120) has four repeated domains of beta-pleated sheets stabilized by cysteines. However these seem to be different from Ig domains. (Figure 12.2a)

2. Most TNFs are juxtacrine factors produced by monocytes and macrophages (Figure 12-2b) composed of extensive β pleated sheets

3. Clusters in trimer upon binding TNFs. (Figure 12.2c)

4. Signal up-regulates immune responses and specifically results in appetite suppression and, long term, cachexia (wasting).
B. Immunoglobulin Superfamily

1. β pleated sheets (bread and butter switch-backs) characteristic of family (Figure 12-3a)

2. Different version bind structurally different ligands
   a. c-Kit (CD117) binds stem cell factor (also called c-kit ligand or steel), Figure 12-3b
   b. IL-1 – general inflammatory up-regulator, Figure 12-3c)

C. Hematopoietin (Class I) – Includes the IL- receptor, one of our major structural examples. (Figure 12.8)

1. largest class of immune-related signal systems. Dozens of specific versions, including receptors for IL-4 and 5 (important in T\textsubscript{H}2 response), G-CSF (important in any defensive response) and receptors for peptide hormones that are not even part of the immune response.

2. function as heterodimers or trimmers. Generally no one part functions optimally in any role without being bound to the others.

3. Receptor has four conserved cysteines at the N terminal domain (CCCC motif), a conserved sequence of tryptophan-serine-whatever-tryptophan serine (WSXWS motif), and a C terminal cytoplasmic signaling tail in both dimers. If there is a third peptide, it aids ligand binding, and does not have these features. (Figure 12-4a)

4. Recruit cytosolic tyrosine kinases

5. Ligand a small monomer with 4 αhelical domains
D. Interferon (Class II)

1. includes receptor for IFN γ, a major inflammatory signal. Also IFN α and β, IL-12 and a dozen or so more. We’ll look at this signaling pathway in detail. (Figure 12.5a)

2. also have four conserved cysteines, distributed in N terminal domain (CCCC motif) and cytoplasmic signal domain, but not the WSXWS motif.

3. function as heterodimers (more rarely trimers)

4. Recruit cytosolic tyrosine kinases and signal via JAK-STAT pathway, a very common sequence in all forms of cell signaling, not just immunity.

5. Ligands are dimers, and two halves bring together two receptor units.

E. IL-17 Receptor Family

1. Most recently identified, with many structural variations.

2. Units have elements similar to TLRs as well as a fibronectin-like domain.

3. Variations have hetero and homodimers and trimers.
4. IL-17 paracrine factor is a small ovoid dimer with beta pleated sheets. (Figure 12.6b)

5. Specifically identified as secreted by a class of T\textsubscript{H} cells, the T\textsubscript{H} 17, which operate at the border of innate and adaptive immunity to trigger a defensive response.

6. Works in boundary defense and against extracellular pathogens

![Figure 12-7](a) 7-span receptor with G protein  (b) chemokine

F. Chemokine Receptor

1. 7-span receptor (Figure 12-7a)

2. associate with G proteins

3. binds chemokines, for example IL-8, which are smallish (90 to 130 amino acids) proteins with alpha helix and beta pleated sheets stabilized with conserved cysteines important in directing traffic during inflammation (Figure 12-7b)

4. Cross reception common: a given chemokine may bind more than one receptor and a specific receptor may bind more than on chemokine.

III. Specific Examples of Signaling Pathways

A. IF\textsubscript{Ny} Signaling via the JAK-STAT Pathway. This is used as a T\textsubscript{H}1 signal to both B cells and macrophages, coordinating the innate and adaptive response to acute infection. While it uses a similar signal transduction to the pathway above, the receptor and specific internal pathway differs.
1. IFNγ is an interferon peptide that can signal
   a. cells to resist viral infection
   b. B cells to class switch to some of the g heavy chains
      (complement-activating IgG3 and IgG1)
   c. Up-regulation of phagocytes and activation of ability of macrophages to present antigen
   d. specific development of Th cells to the Th1 response.

2. The receptor is composed of α and β subunits. JAK kinase bound to the cytoplasmic part with kinase activity on the other side, α and β subunits binding JAK 1 and 2 respectively

3. INFγ dimer binds outside the cell the α and β subunits, causing them to associate.

4. The JAKs phosphorylate each other’s cytoplasmic receptor tyrosines, without recruiting an additional kinase. (Figure 12.16)

5. The receptor now provides a docking site for a STAT protein (potential transcription factor).

6. JAKs then phosphorylate the STAT, activating it.

7. STATs leave the receptor and dimerize.

8. Dimer gets a serine phosphorylated (different cytoplasmic enzyme).

9. STAT enter nucleus and binds to promoters of interferon promoted genes.
B. IL-2: fundamental to adaptive signaling

Check out this animation showing stimulation pathway


1. Structure: The complete activated receptor is a heterotrimer: (Figure 12.9)
   a. \( \alpha \) subunit (CD 25) – structure unrelated to other subunits, aids binding, but doesn’t bind IL-2 very well by itself.
   b. \( \beta \) subunit – signal transduction, CCCC and WSXWS motifs. No binding by itself.
   c. \( \gamma \) subunit - signal transduction, CCCC and WSXWS motifs. No binding by itself, but with \( \beta \) subunit can do some so-so binding.

2. Binding
   a. Resting T cells (except for T\(_{reg}\)) do not have the functioning \( \alpha \) chain (CD 25)
   b. Activation causes synthesis of the \( \alpha \) chain (one of the early genes up-regulated by the immediate genes)
   c. \( \gamma \) chain is constitutively expressed, but cannot bind and signal on its own.
   d. \( \beta \) and \( \gamma \) chains together can bind and signal, but the intermediate affinity usually doesn’t leads to a productive signal. Found in NK cells and resting T cells.
   e. All three together and the overall receptor has high affinity and active signaling. Found in active T\(_H\), T\(_C\), and B cells.

Figure 12-9 IL-2 Receptor

Figure 12-10 IL-2 Receptor (a) with JAKs (b) plus STATS
3. JAK-STAT initiates activation of several internal signaling pathways
   
a. JAK (Janus- or Just Another Kinase) allows β and γ

b. STATs (Signal Transducer and Activation of Transcription) bind, receive phosphates, activate and turn on genes. Several different STATs get activated

c. which allows the signal to up-regulate a variety of different immune genes. (Figure 12.10)

d. “Be fruitful and multiply” signals- IL-2 leads to clonal expansion of lymphocytes by promoting cell division via the Ras pathway and blocking apoptosis.

IV. Cytokine Signaling by CD4+ T Cells: T_{H1}, T_{H17}, T_{H2}

A. T_{H} subsets: T cells with CD4 (T_{C} and T_{Vδ} set aside before decision to use CD4)

1. T_{H1} - cell mediated, activates for acute attack on active bacterial and viral infections and cancers. Signals B cells to make opsonizing (complement activating) IgG3 and IgG1 antibodies. May promote excessive inflammation and tissue injury (scorched earth- sort of life Russia in WWII.).

2. T_{H2} – humoral – defends body against entrenched enemies, especially helminth parasites. Activates eosinophils and mast cells. Signals B cells to make lots of IgM, non-opsonizing IgG and IgE. T cells secrete IL-4 and IL-5. Inappropriate response produces allergy.

3. T_{H17}- inflammatory, and seems to bridge adaptive and innate responses. Secrete TNFα, but not IFNy. Seriously hostile war against something not inside your borders (sort of like US and England in WWII). However, when it goes astray it CAN trigger autoimmune reactions

   a. Cells secrete IL-17 as well as respond to it.

   b. Mobilizes neutrophils and prompts secretion of antimicrobial factors by epithelia.

   c. The pathway triggered in the cells receiving IL-17 is not the JAK-STAT, but rather a completely different pathway resembling the one used by the innate system TLRs. Both activate the transcription factor NF-κB.

   d. Interestingly γδ T cells and NKT cells, which both respond to lipids, protect epithelia, bridge the innate and adaptive systems, secrete IL-17s.
4. **T_{reg}** – suppress immune responses

**B. Summary Table 12-1, CD4+ Cells Compared**

<table>
<thead>
<tr>
<th>T_{H1}-mediated Response (cellular)</th>
<th>T_{H2}-mediated Response (humoral)</th>
<th>T_{reg}-mediated Response (inhibitory)</th>
<th>T_{H17}-mediated (epithelial)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute infection (viral, bacterial, and intracellular pathogens) and inflammation</td>
<td>Chronic or parasitic infection, allergy</td>
<td>Modulation of inflammatory responses, prevent allergy</td>
<td>Acute infection (extracellular bacteria and fungal), opposes T_{reg}</td>
</tr>
<tr>
<td>Moderately strong antibody response, especially IgG1 and 3, which activates complement and phagocytes</td>
<td>Very strong antibody response, especially IgE, also IgM and non-complement activating IgG4</td>
<td>Can attack B cells, reducing antibody production</td>
<td>Inflammatory and humoral response (IgA) while blocking CD-8 cells</td>
</tr>
<tr>
<td>Stimulates development and activity of Tc cells and macrophages (up-regulates B7)</td>
<td>Stimulates development and activity of mast cells and eosinophils</td>
<td>Activates transcription factor: winged helix Foxp3, leading to production of immune suppressing T_{reg} cells</td>
<td></td>
</tr>
<tr>
<td>secrete IFN_{γ}, IL2, TNFβ and TNFα</td>
<td>T_{H2} cells secrete ILs-3, -4, -5, -6, -10, -13</td>
<td>Secrete IL-10 and TGFβ</td>
<td>Secrete TNFα, IL-6 and IL-17</td>
</tr>
<tr>
<td>Activated by macrophages and dendritic cells. These uses PAMPs to recognize pathogens and then signal with ILs</td>
<td>Activated by mast cells, basophils, and NK cells</td>
<td>Activated by resting thymus epithelial cells and resting dendritic cells. Blocked by TLR (inflammatory) pathways</td>
<td>Activated by dendritic cells with activated bacterial PAMPs</td>
</tr>
<tr>
<td>T_{H1} development promoted by IL-3, GM-CSF, IL-12, -18, IFN_{γ} from macrophages</td>
<td>T_{H2} development promoted by IL-3, GM-CSF, IL-4</td>
<td>Promoted by IL-2: express CD25 (IL-2 receptor), promoted by TGFβ and retinoic acid, blocked by IL-6</td>
<td>Promoted by TGFβ with IL-6 blocked by retinoic acid</td>
</tr>
<tr>
<td>Excess response - inflammatory damage to normal tissue (autoimmunity)</td>
<td>Excess response - allergy</td>
<td>Excess response - survival of tumors or infected cells</td>
<td>Autoimmunity (although often different from T_{H1} diseases)</td>
</tr>
</tbody>
</table>

V. Determining Development and Function of T_{H} Subsets

A. Review Figure (Figure 12-11) Notice that all of the defensive sets have up-regulated some form of STAT transcription factor, although each has a different version. Note that this figure presents the agents that act to produce the various cells, and not the cytokines subsequently secreted by them.
1. This is a reminder that variation of the JAK-STAT pathway will have different receptor-ligand triggers and different STAT targets.

2. T regulatory cells have a different role and different controls (Figure 12.21)
   a. CD4+ and CD25+ from the get-go, meaning that they can act faster and to lower levels of IL-2 than the defensive T cell types.
   b. Also associated with class switch to non-inflammatory IgA, which is produced copiously whether you’re experiencing an infection or not (Figure 12.13a)
   c. Down-regulating T<sub>reg</sub> function may prove an important improvement in cancer therapy.
   d. Up-regulating T<sub>reg</sub> cell production may help in treating allergies and autoimmune responses

Figure 12-11 CD4+ T Cell Development

Figure 12-12 T<sub>reg</sub> cell cartoon

Figure 12-13 Immunoglobulins (a) IgA (b) IgE
B. Summary of types of interactions

1. Pleiotropy (Figure 12-14) - one cytokine may act on several different cell types. For example IL-4 coordinates a T<sub>H2</sub> response by acting on B cells and mast cells, promoting cell division in each type. IL-3 and GM-CSF both promote development of T<sub>H1</sub> and T<sub>H2</sub> cells.

![Figure 12-14 Pleiotropy](image)

2. Redundancy (Figure 12-15) - more than one cytokine will produce the same effect. For example, ILs -2, -4 and -5 each, by themselves, induces proliferation in B cells. Different chemokine signals may cross-react with a variety of receptors, and any one will up-regulate the reaction.

![Figure 12-15 Redundancy](image)

3. Synergy (Figure 12-16) - when combinations of stimulus have effects neither has by itself. For example IL-4 and IL-5 together will cause B cells to class switch and make IgE immunoglobulins.

![Figure 12-16 Synergy](image)
4. Antagonism (Figure 12-17) - one cytokine blocks the effects of another. For example, IFNγ (a T<sub>H1</sub> promoter) blocks the ability of IL-4 (a T<sub>H2</sub> promoter) to induce class switching to IgE. Retinoic acid and IL-6 mutually oppose each other in the decision to go T<sub>reg</sub> or T<sub>17</sub>.

5. Cascade induction (Figure 12-18) - one cytokine stimulates other cells to produce a different cytokine, which stimulates yet more cells, etc. We saw that above with the macrophage → IL-12 → T<sub>H1</sub> → IFN-γ → activated macrophage pathway of development. Ongoing macrophage – TH cell conversations are critical to determining the specific tweaking of the immune response.

VI. Clinical Applications

A. Leprosy

2. Not particularly contagious; most people's macrophages can fend it off. Rather benign really: can't migrate on its own, doesn't produce nasty toxins.

3. In some people, however, it survives in within phagosomes of the macrophages. Depending on how the immune system responds, the disease can take one of two courses, or, for that matter, intermediate versions.

4. Generally treatable by antibiotics (rifampicin) and (more recently) thalidomide.’

5. Tuberculoid form - more typical course, resulting from a $T_H1$ response with delayed hypersensitivity.
   a. The host's cell-mediated response leads to formation of granulomas, walled-off regions of infected macrophages.
   b. Most of the bacteria are destroyed by this process.
   c. Skin and nerves are damaged slowly over a period of time.
   d. Patients survive over the long term, but with some nerve damage and possible deformity

Figure 12-19 *Mycobacterium leprae*  
Figure 12-20 granuloma

6. Lepromatous form – $T_H2$ response - this is the nasty version with the bad reputation
   a. Humoral antibodies are released, often at excessive levels.
   b. The bacteria continue to thrive, hiding in the macrophages.
c. Infection disseminates widely beneath the skin and throughout the body; destroys bone cartilage and nerves, and can kill you.

d. Bacteria tend to prefer cooler temperatures, which is why infected individuals tend to lose fingers, toes, ears and noses. More evidence that fever is helpful in fighting disease.

B. Bacterial Septic Shock (as opposed to toxic shock, cause by superantigens)

1. Caused by overproduction of cytokines (general problem discussed previously under superantigens).

2. Gram negative bacteria - recall these are the ones that have a second membrane external to the plasma membrane and thin cell wall and usually a capsule (mostly acidic polysaccharides with some protein) as well.

3. Shock generally refers to a dangerous drop in blood pressure. In the case of bacterial septic shock, there is also high fever, diarrhea and possible blood clotting in various organs. Also, the drop in blood pressure stems primarily from a vasodilatation of peripheral blood vessels (although dehydration doesn't help).

4. The endotoxins from the outer membrane and wall overstimulate the macrophages via the toll-like receptors.

5. The macrophages release too much of the cytokines IL-1 and TNF-a. Mechanism similar to toxic shock.

6. TNF-α all by itself can induce toxic shock in mice.

7. Possible improvements in therapy may involve monoclonal antibodies to these cytokines.

C. Chagas' Disease

1. Caused by *Trypanosoma cruzi*, a member of the trypanosomes which cause a number of other nasty diseases, including African sleeping sickness and related *Leishmania donovani*.

2. Unicellular eukaryotes - protozoans often using various insects as vectors to transfer agents back and forth from people and cattle and other domesticated mammals.

3. The group is generally good at changing surface antigen - one member has over 100 genes coding for surface proteins.
4. *T. cruzi* is a particularly nasty version, living in the guts of insects that live in adobe houses. The insects come out, suck blood from the residents and void feces, which allow the trypanosomes to enter through the break in the skin.

5. Once in the body they multiply and produce an unknown diffusible substance that blocks the $\alpha$ subunit of the IL-2 receptor.

6. The suppression of IL-2 signaling prevents the $\text{T}_\text{H}$ cells from multiplying and signaling to the rest of the system.

7. The parasites then go on to destroy a variety of tissues, including heart muscle, skeletal muscle and nerves. If they’re lucky, people die of heart failure. If they’re unlucky, they die of peristaltic failure due to damage of the nerves coordinating the movement of gut muscles.

8. Treatment:
   
   a. Hard to treat - drugs are dangerous and uncertain.
   
   b. Heart transplants are not an option, as the immune suppressants allow the parasites to kill the patient.
   
   c. A disease of poverty, preventable by better housing.

References:


http://www.oneworldhealth.org/diseases/chagas.php
Lecture 13

The Inflammatory Response

“The fact that there are unsolved problems within the framework of an existing theory does not of itself imply that the theory must be thrown away, or replaced by another; unsolved problems are the essence of science, the means by which theories are refined.” John Maddox in Nature, 1986, vol. 320. April 17, 1986.

I. What, Another Metaphor?!?

A. Full Red Alert

1. When the body perceives a threat, it directs defenses to meet that threat. This means that resources used for growth, physical activity and even higher mental function may be diverted, depending of the severity of the threat. This can save your life.

2. However, if you are on extended alert or full lockdown) for a long period of time, things don’t get done that need to get done. This is comparable to the dangers of chronic inflammation.

B. Roman Insights. The military surgeons of the Roman were the best in the world of their time. Soldiers retired with a full pension at age 40 and the majority lived to collect it, thanks in no small part to the care they received if they were injured. The following are specifically attributed to Celsus and Galen:

1. Hallmarks of the inflammatory response:
   a. rubor (redness)
   b. tumor (swelling)
   c. calor (heat at tissue site)
   d. dolor (pain)
   e. functio laesa - loss of function

2. Cause by vascular changes
   a. endothelium leaks
   b. edema
   c. positive feedback loop of cell signaling
   d. further changes in cell adhesion molecules on both cells and endothelium.
3. Medical Background

a. Long-time recognition that fever accompanies infection. Lower fever, lower infection?

b. Bleeding lowers fever.

c. Giving blood is associated with lower heart disease (as are statins, aspirin and fish oils, all of which are anti-inflammatory and all of which tend to increase bleeding).

II. Signals – This sections reprises and extends material we introduced in Part 1 Lecture 3

A. Pattern Recognition

1. PAMPS (pattern-associated molecular proteins) - generally used to describe a variety of molecules used to identify pathogens. The first group identifies was the toll-like receptors, but many more types.

2. DAMPS (death or damage-associated molecular proteins) - molecules that are normal cell components but should be inside – cytoplasmic or nuclear proteins of membrane proteins belonging in the inner leaflet.

B. Response:
1. Activate complement, which in turn releases inflammatory signals (anaphylatoxins)

2. Attracts neutrophils, macrophages and mast cells.

C. Inflammatory Chemokines

1. Smallish (90 to 130 amino acids), with conserved cysteines

2. Receptors are seven-span membrane proteins. Cross reception common: a given chemokine may bind more than one receptor and a specific receptor may bind more than one chemokine

3. Binding activates G-proteins
   a. large (heterotrimeric with αβγ subunits) – kicks off cytoskeletal changes – quick change in adhesiveness
   b. Switches from active to inactive as it binds GTP and then hydrolyzes it to GDP

4. G-proteins kick off several internal cascades:
   a. hydrolyzes membrane phospholipids to IP3 and DAG
   b. lets Ca^{2+} loose in the cytoplasm, polymerizing actin and promoting movement
   c. kicks off Ras cascade that leads to activation of transcription factors.
D. The High-endothelial Venules

1. found at the end of capillaries in lymph nodes, Peyr’s patches, and tonsils (not spleen)

2. The endothelial lining here is composed of cells that do not look like flattened paving stones, but are thicker.

3. Most lymph cells that extravasate attach to these specific cells.

4. The HEVs specifically attract lymphocytes and do NOT attract neutrophils.

5. The HEV develops in response to foreign antigen exposure: you don’t see this in mice raised in a germ-free environment and you don’t see this in tissues that have the circulation to them blocked to that antigen does not enter.

6. The unique morphology is associated with the induced expression of specific selectins, mucin-like, and Ig CAMs, and they specifically allow different classes of lymphocytes to home to different organs and regions of the body. Examples in the next section.
III. Extravasation

A. Cell Adhesion Molecules - Selectins and Mucins

1. Selectin Family, representation and ribbon diagram of lectin domain.
   a. membrane glycoprotein with lectin at amino terminus that binds carbohydrates. Compare with complement lectin pathway
   b. specific for sialic acid (mucins have a lot of these)
   c. comes is L (Leukocyte), E and P (endothelium) versions
   d. initiate initial sticking of leukocytes to the endothelial wall

2. Mucin-Like Family
   a. protein part rich in serine and threonine (OH-containing R groups)
   b. LOTS of carbohydrate linked to these OHs.
   c. Carbohydrates have lots of sialic acid, interact with selectins
   d. The mucin-like versions of the endothelium interact with the selectins on the leukocytes and vice-versa.
B. Cell Adhesion Molecules- Igs and Integrins

1. I-CAM - Immunoglobulin Super-Family
   a. endothelium has several versions
   b. mucosa has another, which also has a mucin-like domain
   c. bind to integrin on leukocytes
   d. inflammation increases their expression

2. Integrin Family – varied binding partners
   a. Heterodimer (α and β chains)
   b. Expressed in leukocytes
   c. Different integrins bind to different immunoglobulin CAMs (cell specificity)
   d. Can also bind to fibronectin
   e. Cytoplasmic portion can interact with cytoskeleton and signaling proteins such as the tyrosine kinases, Fyn and Lck.

C. Sequence
1. rolling - attachment by low affinity P selectins (yellow) on the endothelium to mucin-like molecules on the neutrophil (purple). Because the connections are loose, they tend to break and the neutrophils kind of roll along, attaching to one endothelial cell after the next as it is swept along by the flow of blood.

2. activation – the “stick and release” from the rolling response along the endothelium triggers chemokine (IL-8) release by the endothelium. The neutrophils activate G-protein cytoplasmic pathways via the 7-span receptor.
   
a. Video shows lipid raft. We looked at these in connection with T cell activation, but they also form membrane compartment to organize other interactions as well. Will return to this.
   
b. The mechanical pulling and tugging causes the endothelium to release a chemokines.

3. arrest and adhesion - The G-proteins activate integrins, changing their conformation, and increasing their affinity for Ig-related CAMs. This nails down the neutrophils. Neutrophils cannot bind to non-inflamed endothelium.
   
a. The chemokine binds to a 7-span chemokine receptor.
   
b. This receptor activates a G protein and sets off an internal signaling cascade.
   
c. The integrins change conformation, enter the lipid rafts, deploy and stick Ig tightly to the Ig CAMs of the endothelium.
d. Some of the first proteins affected are those that associated with the inner side of the plasma membrane and connect to the cytoskeleton.

4. Transendothelial migration - the arrested neutrophil then finds the gap between two adjacent endothelial cells and squeezes through it.
   
a. The cytoskeletal changes lead to the neutrophils changing shape and moving by amoeboid motion.
   
b. The neutrophil forms a leading wedge and ootches through a gap between the endothelial cells (recall that inflammation makes the endothelium somewhat leaky).

D. Lymphocyte Extravasation, Trafficking, and Homing

While the processes that allow the lymphocyte to leave the blood vessel (rolling, activation, arrest, and migration) are similar, the mechanisms that control exactly where they lymphocyte will undergo extravasation are more complex and involve specific homing signals.

1. Naïve lymphocytes

Lymphocytes migrate in and out of secondary lymph organs where they contact presenting sentinel dendritic cells. Recall that the chance of any one lymphocyte recognizing any one antigen are miniscule (1 in $10^5$), so the cells run repeated loops through secondary organs and in and out of circulation, essentially kissing frog after frog and looking for the rare prince.

a. T cells, for example are attracted by inflammatory signals and cruise into sites of active infection, where they may be activated by antigen presented by innate cells fighting the infection.

b. T cells may also be activated by dendritic cells in the lymph nodes in spleen. In that case the dendritic cells also communicate the site of the infection by activating specific T-cell surface receptors for cytokines and CAMs characteristic of particular tissues. For example retinal retinoic acid is produced by the gut and only gut-homing T cells have receptors for it. For skin, it’s vitamin D.

2. Effector and Memory Cells

a. Effector cells tend to head to the site of infection.

b. Memory cells tend to head to the tissues that initially had the infection.
c. Cells are directed by a combination of cytokines and cell surface receptors (above) but can also be retrained, which is necessary if an infection spreads.

d. T cells, in particular, are programmed to home to a specific site because they express a specific combination of cytokine and CAM receptors.

IV. Inflammatory Signaling Network

A. Inflammatory Cytokines – initiating inflammation

1. IL-1 - comprises a family of at least 11 related signaling molecules using Ig receptors.
   a. The signals are smallish proteins with β-pleated sheets.
   b. Synthesized as precursor in the cytosol, then clipped and secreted: leaderless secreted proteins.
   c. Most members activate TH17 cells, although an inhibitory version exists as well.

2. IL-18 – also a member of this general family, but with different controls and targets.
   a. Synthesized in the cytosol, clipped and secreted.
   b. Production of precursor under different controls.
   c. Activate TH1 responses.
   d. Promote IFN-γ production, which in turn promotes IL-18 secretion in a positive feedback loop.
B. PRRS- Release of IL-1 subtypes and IL-18 are in turn promoted by activation of pattern-recognition receptors. Along with complement activation, the signaling that leads to IL-1 and IL-18 release is part of the very first responses to a new infection. Here are two examples:

1. LPS (lipopolysaccharide) activation of TLRs – recall that these TLRs are transmembrane proteins with a cytoplasmic domain that kicks off a complex pathway to NFkB and AP-1 activation, which in turn up-regulate genes for inflammatory cytokines: IL-1, IL-18, IL-6 and TNF α.

2. NOD receptors (members of a group of NLR proteins with annoying complex naming conventions) are in the cytoplasm, recognize viral, bacterial and parasite signatures using a leucine-rich repeat domain, as well as DAMPS and environmental irritants. Also activate NFkB and AP-1 and thus inflammatory cytokines.

C. Activation (again, this is a simplified example)

1. Pattern Recognition Receptors, via NFkB and AP-1, upregulate IL-1 production in the cytoplasm. IL-18 constitutive, but still requires this as activating signal.

2. Further stimulus (a second signal) by other danger triggers activated assembly of inflammasome, a complex of proteins.
   a. Pathogenic signatures – wide variety of proteins, nucleic acids and cell wall components from bacteria, virus, fungi and protozoa.
   b. Environmental irritants- alum (used as a vaccine adjuvant), inorganic particles (asbestos, silica, metals), UV light.
   c. Internal signals- ATP and glucose, crystals of cholesterol and urate, β-amyloid, hyaluronin.

Figure 13-13, NLR activation
3. NLR proteins (NLR-P3 as an example) receive signal at the leucine-rich and shift. Since a huge variety of signals are effective here, the activation does not seem to occur by a classic ligand-receptor interaction.

4. Conformational shift also recruits associated ASC with CARD domain

5. ASC recruits caspase-1 (yes, of apoptosis fame) orienting the hydrolytic domains toward the center, axle region

![Figure 13-14, Inflammasome assembling and activating](image)

6. Caspase 1 undergoes hydrolysis, activates and forms an active region at the hub.

7. Caspase-1 clips IL-1 precursor.

8. Part goes to nucleus of the cell (positive feedback loop)

9. Part is secreted from the cytoplasm

   a. TLR LPS signal necessary for secretion, too.
   b. Precursor enters secretory lysosome and get secreted.
   c. Co-opted mechanisms used to turn over organelles and proteins and to secrete proteolytic enzymes

10. May have originally evolved from leakage from dying cells, but is clearly a controlled process today.
D. Inflammasome Structure in Context.

Inflammasome structure is a complex and interesting assembly of complex and interesting proteins, not bound or associated with a membrane, that assembles and in response to danger signals in the cell and can disassociate and recycle.

Figure 13-15, Apoptosome assembly

1. The apoptosome is a similar looking structure that also activates caspases.

2. Similarities-
   a. wheel-like structures, typically seven units
   b. homologies in regions of the axis/assembly
   c. activated by leucine rich ligand binding domains
   d. have CARD (caspase recruitment) domains at axis
   e. bind caspases via their corresponding CARD domains
   f. Caspases active hydrolytic domains at axle region
   g. Caspases then clip effector proteins

3. Differences-
   a. Basic structural proteins
   b. Activation trigger
   c. end result
Table 1- Inflammasome and Apoptosome Compared

<table>
<thead>
<tr>
<th>Name</th>
<th>Inflammasome</th>
<th>Apoptosome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Structural protein</td>
<td>NLR (NOD-like leucine-rich receptors)</td>
<td>Apaf-1 (apoptosis protease activating factor)</td>
</tr>
<tr>
<td>Activator (ligand)</td>
<td>Various danger signals</td>
<td>Cytochrome c</td>
</tr>
<tr>
<td>CARD (caspase recruitment domain)</td>
<td>Recruited- (ASC) adapter protein</td>
<td>Part of Apaf-1</td>
</tr>
<tr>
<td>Adds at axle and activates</td>
<td>Caspase 1 (with CARD domain)</td>
<td>Caspase 9 (with CARD domain)</td>
</tr>
<tr>
<td>caspase activates by clipping precursor</td>
<td>IL-1 or IL-18 (secreted and signal inflammation)</td>
<td>Executioner caspases (break up cell components)</td>
</tr>
</tbody>
</table>

V. Inflammatory Mediators

A. Clotting

Central to dealing with barrier damage and raising the alarm at the first sign of a breach.
1. Clotting both up-regulates, and is up-regulated by, inflammation. This is why chronic inflammation can lead to strokes and cardiac infarcts.

2. Initiated by platelets and RBCs contacting damages surfaces (collagen fibrils) and breaking open.

3. This releases and activates Hageman factor, a large heterodimer plasma protein with hydrolytic activity (serine protease.)

4. Activated Hageman factor (sometimes called Factor XII) initiates an expanding hydrolytic cascade.

5. The clotting cascade ends with prothrombin activation to thrombin, which clips fibrinogen into fibrin and peptides.

6. The fibrin forms the clot, trapping red blood cells.

7. As the wound heals, a system of fibrinolytic enzymes producing plasmin dismantles the clot, using the enzyme plasmin.

8. The released peptides promote inflammation. Plasmin also produces inflammatory peptides and activates complement.

9. Hageman factor helps activate this process.
B. Kinins – Signals Derived from Peptides

1. Bradykinin – 9 amino acid peptide specifically leads to pain, in addition to increased endothelial permeability and smooth muscle contraction. This may not look like a peptide, in part because it contains prolines at position 2, 3, and 7, which distort the peptide backbone.

2. Cascade initiated by Hageman factor, again by proteolysis, eventually activating kallikrein, which turns around and activates more Hageman in a positive feedback loop.


4. ACE inhibitors block the breakdown of bradykinin, lowering blood pressure (but for some reason not increasing pain.) this happens as a side effect of blocking a different signaling pathway.

C. Lipid derivatives- these often depend of activations of specific enzymes.

1. Membrane phospholipids play a structural role but also serve as the raw material for generating a lot of signaling compounds.

2. Phospholipids have two fatty acids linked to glycerol and the glycerol links up via a phosphate to a polar group (ethanoleamine or inositol for example).

3. Depending on what you begin with and where you cut it, and how you modify the results, you can get an array of different potent signaling molecules.

[Diagram of membrane phospholipid]

Figure 13-21, membrane phospholipid figure 13-22, phosphoinositol

4. Inositol-containing phospholipid

5. Clip to DAG and PIP3, used in previous pathways.
6. A different clip, can remove a free fatty acid.

7. Arachidonic acid - derived from one to the fatty acids.

8. Act on it with enzymes called cyclooxygenases and you kind of bend it into the hairpin shape.
   
   d. Produces thromboxane or a variety of prostaglandins
   
   e. Inhibited by COX2 inhibitors block cyclo-oxygenases and block inflammation and NSAIDs (aspirin, Advil) will also block prostaglandins

9. Act on it with lipoxygenase and get a variety of leukotrienes.
   
   a. Released by mast cells in allergic response.
   
   b. Antihistamines have been joined by anti-leukotrienes in over-the-counter (OTC) medication.

10. In a separate origin pathway, phospholipids can provide the raw material for Lyso-PAF

   a. Phospholipid in this case has ethanolamine polar group.
   
   b. One fatty acid
   
   c. Remains of the second, which used to be attached in the middle of the glycerol.
   
   d. Now have PAF (Platelet Activating Factor)
   
   e. Inhibitory drugs under investigation as for treating both allergies and cardiovascular problem.
VI. Clinical Considerations- All of this signaling and enzymatic activity provides a lot of opportunity of diagnosis, monitoring and treating disease.

A. Disease

1. Systemic Acute-Phase Response – defense from something like flu or bacterial disease – acts more globally- if your toe infection turns into sepsis, for example.
   a. Compromised by malnutrition or starvation
   b. IL-6, TNF, IFN γ
   c. Act on the hypothalamus to induce fever and CRP release, which in turn causes the pituitary to release of ACTH which causes the adrenals to produce steroids (cortisol etc.).
   d. The liver responds with the release of acute-phase proteins (a general term) - activates complement.
   e. increased leukocyte production and activation, especially neutrophils

2. Chronic Inflammatory Response – IFNγ and TNFα, transcription factor NF-κB
   a. persistent infection (gum disease), autoimmune response, cancer, chronic injury.
   b. old age, obesity, diet high in trans-fats, triglycerides (diet high in sugar) wrong gut flora, diabetes, sleep disorders.
   c. contributes to disease processes, including cancer and cardiovascular diseases
   d. Increased clotting
   e. fibrosis - a type of scar tissue formed when chronic inflammation leads to excess production of fibroblasts and collagen
   f. granuloma (also called tubercle) - an attempt to wall off the problem with macrophages and specialized Th cells.

B. Anti-inflammatory - Braking the System- Hey, that drug sound familiar!

1. Antibodies used to block leukocyte extravasation:
   a. antibodies to integrins
   b. antibodies to CAMS

2. Corticosteroids
   a. Pregnisone, cortisone, dihydrocortisone
   b. High doses block adrenals and many immune functions
3. NSAIDs

a. Aspirin (acetylsalicylic acid), Advil (ibuprofen) Aleve (naproxin) are OTC

b. prevent prostaglandin production from arachidonic acid. Celebrex specifically blocks COX-2

c. NOT Tylenol/acetaminophen- acts on brain to raise pain threshold and lower hypothalamic thermostat).

4. Cooling - Interferes with inflammatory process (recall that fever is often a helpful part of the response to infection)

a. used to prevent excessive tissue damage following injury or stroke-
   exercise raises levels of CRP and other inflammatory indicators

b. used during surgery

c. used to cut down on symptoms of autoimmune disease multiple sclerosis
   – chilling via hands to allow athletic competition.

d. Harmful if you’re ill, unless your fever is dangerously high.

References:

Inner Life of the cell – Narrated: http://multimedia.mcb.harvard.edu/innerHi.swf  

Leaderless secretory proteins:
http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3662868/

Structure of IL-18 binding complex
http://www.nature.com/ncomms/2014/141215/ncomms6340/full/ncomms6340.html IL-18


WAY beyond the content of this course, but should you become fascinated with apoptosis control:  http://www.nature.com/nrm/journal/v9/n5/box/nrm2393_BX1.html
Lecture 14

Cell-Mediated Cytotoxic Responses

The future is already here. It is just not evenly distributed. - William Gibson

I. Division of Labor. While the primary agents of this form of cell-mediated (TH1) immunity are the Tc and NK cells, other cells make important contributions to the process.

A. Lymphoid cells developing in the thymus – rearrange TCR genes

1. TH - signal to tailor and coordinate responses.

![Figure 14-1 CD4+ T cells (a) TH (b) Tc](image1.png)

2. TC – attack and kill cells displaying specific antigens by disrupting membrane and lysing cell.

3. γδ chain T cells - patrol epithelia, attack and kill pathogens and rogue-self using same mechanisms as NK and Tc cells, signal. Some authors refer to these as “primitive” because their function seems less specialized.

![Figure 14-2 T Cell Variants (a) αβ and γδ (b) NKT](image2.png)
4. NKT- innate recognition – iNKT cells (type 1) respond to lipid antigens, especially those characteristic of bacteria. Other NKT cells (type 2) recognize a wider array of lipids and have more variation in the receptor.

   a. Type 1 (iNKT) cells produce a semi-invariant $\alpha\beta$ TCR. They always use the same $\alpha$ option, and use one of a limited number of $\beta$ options. There will still be some variation at the regions where you put them together.
   b. These cells develop in the thymus, are CD4+ (or sometime DN) and bind to cells presenting lipids on CD1d. This is a typical $T_H$ function.
   c. has CD 16 (the Fc receptor – a typical NK function)
   d. requires TLR recognition of bacterial responds to activate
   e. attacks bacterial cells with inflammatory cytokine signaling, including IL-17, IFNγ
   f. attacks tumors with specific lipids, using FAS ligand and perforins (a typical NK and $T_C$ function.)
   g. prevents autoimmunity (a $T_{reg}$ and $\gamma\delta$ function), does not form memory cells, and seems to be quasi- innate

B. Lymphoid cells developing in the bone marrow – do not rearrange TCR genes

1. B cells – rearrange immunoglobulin genes, phagocytize, but do not seem to be a primary killer of pathogens, and do not attack rogue self. The antibodies that they produce are often important in directing attack by other cells.

2. NK (natural killer) - do not rearrange any genes. They attack and kill cells with suspicious surface displays by disrupting their membranes and lysing the cells.

(a) B cell   (b) NK cell   (c) $T_{reg}$ cell

Figure 14-3 Cartoon Lymphocytes
C. Context

1. Innate to adaptive spectrum

![Diagram: Lymphoid Cells in Order of Innate to Most Adapted]

2. Developmental Flow Chart

![Diagram: Hematopoietic Stem Cell]

Hematopoietic Stem Cell

↓ GATA 2

pluripotent differentiating cell

↓ ikaros

Lymphoid stem cell

CD44 (target to thymus)

DN T cell → γδ T cell

αβ T cell (positive selection)

CD4

↓

CD8

↓

NKT

Treg

T17

TH

TC

Fundamentals of Immunology, Part 2 -101
D. Myeloid Cells

1. macrophages – phagocytize, present antigen
2. neutrophils – phagocytize, do not present antigen
3. eosinophils – coordinate attack on eukaryotic parasites, do not present antigen, weakly phagocytic

II. Attack of the T<sub>C</sub> cell

A. Activation

1. The T<sub>C</sub> cell binds a foreign antigen on MHC I (T<sub>C</sub> cells that bind self-antigen have been killed off during negative selection.)

2. CD45R on T<sub>C</sub> and T<sub>H</sub> attaches to CD22 (sometimes called CD45L) on target cells.
3. Th cell activates the cell, turning it into a CTL.

4. Cross presentation speeds this process by making it easier for the Tc cells to encounter the foreign antigen.

5. Activation leads to

   a. Cell division
   b. Up-regulation of cell adhesion molecules
   c. Synthesis of the complete IL-2 receptor

Figure 14-7 Display to Tc cells

B. CTLs on the Attack

http://www.youtube.com/watch?v=WPaRKm2-YNY

http://www.youtube.com/watch?v=FWWMOb7z5GI

Figure 14-8 Interaction between Tc cells and self-cells
1. CTL cell attached to target will up-regulate cell adhesion molecules, forming conjugate

2. CTL attacks target membrane
   a. cytoskeleton rearranges, directing Golgi and storage granules (perforins and granzymes) to target cell
   b. granule contents released by exocytosis (Ca^{2+} dependent)
   c. Perforin monomers undergo conformational change and insert in the target membrane (sequence homology with C9 of the complement system).
   d. Granzymes (serine proteases) enter through pores and activate apoptosis.

![Figure 14-9 Apoptosis](image1)

![Figure 14-10 Infected cell downregulating MHC I](image2)

3. Apoptosis can also be induced by Fas ligand binding to target Fas receptor on the target cells
   a. Ipr mice are mutant for Fas (receptor) production.
   b. They laugh at Fas ligand.
   c. Cells from mice that are both perforin knock-out AND Fas knock-out cannot be killed by cytotoxic T cells.
   d. Either pathway initiates apoptosis in target cells.
   e. Ultimately, each sets off a pathway that activates a cascade of proteolytic enzymes (caspases).
Table 14-1, How Mutations Affect Ability to Induce Apoptosis

<table>
<thead>
<tr>
<th>Infected, but otherwise normal, mice</th>
<th>CTLs produce perforin and FAS ligand</th>
<th>All cells respond to FAS ligand</th>
<th>Infected cells die of apoptosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Perforin mutant</td>
<td>CTLs produce FAS ligand, but not perforin</td>
<td>All cells respond to FAS ligand</td>
<td>Infected cells die of apoptosis</td>
</tr>
<tr>
<td>Ipr mutant, does not make FAS (the receptor)</td>
<td>CTLs produce perforin and FAS ligand</td>
<td>Cells do not respond to FAS ligand</td>
<td>Infected cells die of apoptosis</td>
</tr>
<tr>
<td>Double mutant</td>
<td>CTLs produce FAS ligand, but not perforin</td>
<td>Cells do not respond to FAS ligand</td>
<td>Infected cells survive</td>
</tr>
</tbody>
</table>

4. CTL-dissociation
   a. The integrin holding the cells together reverts to its less avid form after 5 to 10 minutes.
   b. The CTL undocks and begins looking for another cell to hit.
   c. The target cell has already received its death signal.

5. Target cell destruction
   a. The target cell undergoes apoptosis.
   b. The pieces are scavenged by the macrophages or neutrophils.

III. Natural Killer Cells – yet another link between innate and immune systems

Figure 14-11 NK cell cartoon

Figure 14-12 NK cell micrograph
A. Characteristics

1. Large cells with lots of granular inclusions.

2. Lymphoid, sharing a common progenitor and surface markers with T cells, including the use of signaling peptides similar to those in the complete CD3 complex.

3. Develop in the bone marrow and do not rearrange genes.

4. Also have TLRs and inflammatory cytokine receptors typical of those of macrophages and neutrophils.

5. Do express the receptor of Fc of IgG (CD 16) and the IL-2 receptor. This allows them to recognize and attack antibody-coated cells during ADCC.

6. Have MHC I receptor, but it is inhibitory, and not used to recognize antigen, but rather viral down-regulation.

7. Pre-programmed to attack cells with surface characteristics that are likely indicators of trouble.

B. Mechanisms of Killing

http://www.youtube.com/watch?v=84MlWh1XN0Q
http://www.youtube.com/watch?v=NdxAlyn73sU

In this one, the NK cell is directed by an antibody on the surface of the cancer cell.

1. Granules containing perforins and granzymes directionally released at target cell.

2. Constitutively cytotoxic; do not require activation.

3. Do not require antigen or MHC recognition.

4. Adhesion to target triggers release.

C. Control of Killing

Because these cells run around in the ready, there are opposing controls that act to either unleash the NKs or rein them in.

1. Class I MHC receptor coupled to AR (inhibitory) receptor. Enough MHC and the AR will inhibit killing no matter what the other signals.
2. However virus that down-regulate MHC I production may fool Tc cells, but they will activate NK cells.

3. Stress receptor. There are a variety of stress indications displayed by sick cells, and a variety of receptors used by NK cells to sense them. The interaction promotes attack.

![Figure 14-13 NK cell interacting with healthy and viral infected cells. In the last two infections the cells have down regulated MHC I and in the last produced an MHC I analog](image)

4. Cytomegalovirus not only lowers the cell's MHC production, it attempts to fool the NK cells by producing a fake MHC. The viral genome codes for a protein resembling MHC I. This does not activate Tc cells as it does not display foreign antigen. However, it is recognized as MHC I by NK cells of susceptible mice. The fake MHC binds to the same NK inhibitory receptor that real MHC binds to.

5. Some mice have evolved NK surface receptors that can distinguish fake viral MHC I from the real thing. These mice have an NK receptor for the viral protein that activates attack, instead of suppressing it. And these mice are quite resistant to CMV.
IV. Coordinated Killing - Antibody Dependent Cell Mediated Cytotoxicity (ADCC)

A. How it Works

1. Targeting of cell harboring pathogens.
   a. The cells display abnormal antigen.
   b. B-cells produce antibodies to this antigen
   c. The antibodies attach so that their Fc portion stick out from the cell.

2. Cells with Antibody (Fc) Receptors
   a. NK
   b. macrophages
   c. neutrophils
   d. eosinophils
Table 14-2 ADCC Variations

<table>
<thead>
<tr>
<th>Cell</th>
<th>Antibody Recognized</th>
<th>Cells Attacked</th>
<th>Result</th>
<th>Other Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>NK</td>
<td>IgG, IgM</td>
<td>rogue self</td>
<td>apoptosis</td>
<td>possible damage to healthy tissues</td>
</tr>
<tr>
<td>Macrophage</td>
<td>IgG, IgM</td>
<td>pathogen</td>
<td>phagocytosis</td>
<td>antigen presentation on MHC II</td>
</tr>
<tr>
<td>Neutrophil</td>
<td>IgG, IgM</td>
<td>pathogen</td>
<td>phagocytosis</td>
<td>often break open</td>
</tr>
<tr>
<td>Eosinophil</td>
<td>IgE</td>
<td>parasites</td>
<td>surface attack</td>
<td>may break open or release toxins</td>
</tr>
</tbody>
</table>

B. Graft Versus Host – Cooperation from the immune system you would like to avoid.

1. Supply immuno-incompetent individual with competent lymphocytes from another genetically different individual.

2. The Tc cells activate and begin to attack the host cells.

3. The B cells produce antibodies to the host cells surface antigen.

4. The patient experiences a cell-mediated attack from both NK and Tc cells, with some help from the macrophages, neutrophils and maybe even complement.

5. The cells under heaviest attack are GI epithelial (diarrhea), skin (which may slough off in sheets), and spleen, (which may attack the red blood cells, resulting in jaundice). The whole response can kill a person.

6. Spleen enlargement is generally used as a diagnostic indicator.

NK cells: [http://www.youtube.com/watch?v=HNP1EAYLhOs&feature=fvwrel](http://www.youtube.com/watch?v=HNP1EAYLhOs&feature=fvwrel)


Leukocytes (eosinophils?) attacking worm: [http://imgur.com/gallery/YQftVYv](http://imgur.com/gallery/YQftVYv)

Glossary

- Adaptive cells – those that rearrange their genes.
- ADCC- antibody dependent cell-mediated cytotoxicity. A process by which white blood cells recognize the stems of antibodies attached to a cell and then attack it.
- Allele- a version of the gene. There are two alleles for the enzyme that produces color in four o’clock flowers, one that codes for an enzyme used to make red pigment and a different DNA sequence that does not produce a functional enzyme, leaving the flower white.
- ALT – associated lymphoid tissue. MALT (mucosal), GALT (gut), BALT (bronchial), NALT (nasal)
- Antibody- a soluble immunoglobulin.
- Antigen- a molecule that can bind to an antibody, B cell receptor or T cell receptor.
- APC – antigen presenting cell. Cells that present antigen on MHC II to T\textsubscript{H} cells.
- Apoptosis – programmed cell death.
- ATP- adenosine triphosphate, directly supplies energy to many biological reactions.
- B7 – an important juxtacrine signal from APCs to T\textsubscript{H} cells.
- BSA – bovine serum albumin. A smallish soluble protein isolated from cow’s blood.
- Calnexin- first MHC I chaperone, displaced by beta macroglobulin.
- Calreticulin- binds to tapasin- MHC I complex
- CAM – cell adhesion molecule. Any one of a number of different molecules that help stick cells together.
- Centromere- the region on a chromosome that attaches to the spindle during mitosis.
- CD – cluster of differentiation. Refers to the isolation of cells by flow cytometry depending on exactly what proteins extend from a cell’s surface. This in turn influences how the cell moves during the separation process. Recall that these are applied solely on the basis of the order of discovery.
CD1 (A through E) - the peptides or genes that code for them that bind lipids to present them to T cells.

CD3 - the peptides that associate with the TCR for signaling.

CD4 - the co-receptor that helps T\textsubscript{H} cells bind to MHC II.

CD8 - the co-receptor that helps T\textsubscript{C} cells bind to MHC I.

CDR - complementarity determining region - the recognition side on the tips of the antibody arms.

Chagas Disease - caused by a trypanosome (unicellular eukaryote) that is spread by an insect vector. Changes surface coats and causes down-regulated IL-2.

Chitin - cell wall material of fungi, also an important component of insect exoskeletons.

Chordate - member of the phylum Chordata. Includes vertebrates and invertebrates with a dorsal nerve cord, gill slits, notochord and muscles in blocks.

Clip - portion remaining after hydrolysis of the invariant chain.

CLP - common lymphoid progenitor. Gives rise to lymphoid cells: NK, T cells, B cells, and more.

CMP - common myeloid progenitor. Stem cell that can give rise to any myeloid cell type (including red blood cells and platelets).

Coley toxin - inflammatory material isolated from bacteria used in cancer chemotherapy around 1900.

Complement - a system of proteins that helps identify pathogens and debris for destruction and phagocytosis (the landmines of the plasma).

CTL - Cytotoxic T cell. Activated T\textsubscript{C} cell, ready to kill rogue-self cells.

DAG - diacyl glycerol. Two fatty acids joined by ester condensation bonds to glycerol, produced by clipping a phospholipid.

DAMPS (death or damage-associated molecular proteins) - molecules that are normal cell components but should be inside the cell, not identifiable on the exterior.

Down-regulate - turn off cellular processes (often a result of negative feedback).

Downstream - the end of the DNA or RNA with the free 3’ carbon of the (deoxy) ribose. Nucleic acid synthesis and translation proceeds 5’ to 3’.
- DN 1 through 4 - stages of T cell development before cells express either CD4 or CD8.

- Early genes - genes for signaling proteins and receptors activated within hours after T-cell activation.

- Epitope – the specific portion of a molecule that binds to an adaptive receptor. For example, a viral protein is an antigen whose different epitopes bind to different antibody idiotypes.

- ERp57- final chaperone binding to MHC I prior to antigen binding.

- Exon- the part of a gene that codes for a sequence of RNA that will wind up in a message and get translated (expressed.) A gene or gene region may have one too many exons.

- FAS/FAS ligand - A tripartite juxtacrine signal set, used by immune cells to induce apoptosis in damaged cells.

- G protein (trimeric) – an important internal signal transducer. It responds to receptor activation by exchanging GTP for GDP and triggering a signal cascade to the cell’s interior.

- Gamma-delta T cells- those with the quasi-innate gamma-delta receptor, involved in boundary defense.

- Gene region – a sequence of DNA coding for a specific part of the Ig or T-cell receptor.

- Goldilocks Principle- a general principle that in many biological system the intermediate value of a process or level or a signal is most effective.

- Granulocytes – Cells with copious granular inclusions and that do not present antigen. Includes neutrophils, basophils, and eosinophils (which have oddly-shaped nuclei) and mast cells (which do not).

- Granuloma (also called tubercle) – a nodule of macrophages and specialized T\textsubscript{H} cells surrounding a walling off macrophages infected with intracellular bacteria.

- Haplotype- a group of genes next to each other on a chromosome that tend to be inherited as a block.

- Hapten- a molecule that could potentially bind a CDR, but by itself is not large enough to kick off an immune response.

- HLA- human lymphocyte antigen, the MHC types of humans.

- HLA-DM – helps load MHC II by displacing clip.
- HSC- hematopoietic stem cell. Can divide and regenerate to develop into any type of blood cell. Found in bone marrow.

- Humoral response – Immune defense found in the plasma, the word humor derived from the ancient Greek medical theory of body fluids; it really just refers to antibodies. Stupid term.

If people stop using it, maybe it will go away.

- Hybridoma- a cell or cell line derived for the fusion of a blood cell cancer (myeloma) and a normal, antibody-producing plasma (B lineage) cell.

- Idiotype – a category of antibodies that all have the same recognition region

- IL (-1, -2, -4, -5, etc.) – interleukins- a general name for a heterogeneous collection of paracrine signaling molecules.

- Immediate genes- T cell activation gene for transcription factors, activated in minutes.

- iNKT cells – quasi –innate T cells that bind to lipids.

- Integrin- proteins that spans the plasma membrane, connecting to the cytoskeleton at the interior and capable of binding a variety of extracellular proteins at the exterior.

- Innate cells – those that do not rearrange genes.

- Introns- that DNA sequences of the gene that code for RNA sequences that get clipped out during processing.

- Invariant chain- MHC II chaperone.

- IP2 or IP3- inositol with two or three phosphates. Produced by clipping inositol from a phospholipid and adding one or two phosphates to it (It has one to begin with).

- Isotype- a category of antibodies of the same class.

- Janus kinase- two-faced receptor. The signaling domains phosphorylate each other during signaling.

- Kinase- and enzyme that adds phosphate to proteins or other molecules.

- Late genes- gene for CAMS up-regulated by T cell stimulus. These take days to activate.

- Leprosy- a relatively non-contagious bacterial disease. An ineffective immune response results in the disfiguring loss of extremities.
· Lipid raft- a patch of plasma membrane with a distinct lipid composition and a more rigid, thicker structure. Helps to separate different functional membrane protein from each other.

· LMP 2 and 7- proteins that determine the peptide length of peptide hydrolyzed in the proteasome.

· Lymphoid cells – white blood cell types (innate and adaptive) found in the lymph and (and blood and immune organs as well).

· MAC- membrane attack complex- terminal complement pathway produces this, which punches holes in plasma membranes.

· MASP- mannose associated protein. Associated with lectin pathway of complement.

· MBL- mannose binding lectin. Associated with lectin pathway of complement.

· MHC- Major Histocompatibility Complex. Includes the genes and the proteins they code for. These include the proteins (groups I and II) that hold small peptides so that T cells can recognize them. They also include a variety of other proteins, including enzymes important in immune recognition and promotion. The human versions are named HLA molecules for human lymphocyte antigen.

· Monoclonal- refers to a cell line of (theoretically) identical cells derived from the division of a single cell.

· Mucin- CAM with proteins attached to extensive amounts of branched carbohydrate chains, ending in sialic acid.

· Myeloid cells – innate white blood cells rarely found in the lymph.

· Necrosis- cell death from disease or injury.

· NFAT- inflammatory transcription factor activated by phosphatases in turn activated by MAP kinases.

· NFκB- inflammatory transcription factor activated by phosphatases in turn activated by MAP kinases.

· NK cell- Natural Killer cell. Kills rogue-self cells, recognizing them by innate mechanisms. Does not require T_H activation.

· N-nucleotide addition- During gene rearrangement, when enzymes add nucleotides at random in the palindromic regions of the joint.

· NOD – nucleotide oligomer detectors. Soluble pattern recognition receptors found in the cytoplasm of cells. Despite the name, they often recognize cell wall materials.
- NSAID - non-steroidal anti-inflammatory.
- Nucleic acid - RNA or DNA.
- PAMPS (pattern-associated molecular proteins) - generally used to describe a variety of molecules characteristic of proteins recognized by PRRs such as TLRs.
- Peptidoglycan - mesh-like macromolecules that compose the basic structure of the bacterial cell wall.
- Perforins - monomer secreted by NK and Tc cells that assemble into pores that construct holes in the plasma membranes of self-cells under immune attack. Resemble the pores produced by complement C9.
- Phagocytosis - when a cell engulfs large particles.
- Pinocytosis - when a cell gathers fluid in a vesicle and engulfs the vesicle.
- Plasma cell - activated antigen-secreting B cell.
- P-nucleotide addition - During gene rearrangement, when enzymes fill in the missing nucleotides at the joint by copying the palindromic nucleotides on the other strand.
- Proteasome - organelle responsible for hydrolyzing and recycling cytosolic proteins.
- Receptor-mediated endocytosis - when a cell binds material at its surface using a proteins receptor and then internalized the complex into a vesicle that enters the cell.
- RAG enzymes - those responsible for gene rearrangement (related to gene used during meiosis for gene recombination).
- RSS - recombination signal sequence. The sequence of 28 or 40 nucleotides that the upstream or downstream end of a gene region providing the signals for gene rearrangement.
- Selectin - class of CAMS that have a lectin domain that bind to mucins carbohydrates.
- Simple sugar - single sugar unit, includes glucose, mannose and galactose. May be modified into sugar units as sialic acid (NANA) or N-acetyl glucosamine.
- STAT - transcription factor activated by phosphorylation and subsequent dimerization (Signal Transduction Activator of Transcription). Typically activated by Janus kinases.
- TAP 1 and 2 - transport peptides into the ER using ATP.
· Tapasin- bring TAP to MHC I.
· T<sub>C</sub> cell- cells that recognize rogue self-cells by antigen they present on MHC I. They develop into CTLs after instructions from T<sub>H</sub> cells.
· TCR – T-cell receptor. Found extending from the surface of both T<sub>C</sub> and T<sub>H</sub> cells. Recognizes antigen, coded for by rearranged genes.
· T<sub>H</sub> cell- thyroid helper cell. Coordinates immune responses. T<sub>H</sub> 1 cells promote a serious response; T<sub>H</sub> 2 promotes a containment response, T<sub>H</sub>17 promotes inflammation and boundary defense, and Treg tolerance. There are additional types as well.
· TLR – Toll-like receptors. Pattern recognition receptors that recognize molecules characteristic of pathogens. Found embedded in plasma membrane and endomembranes of many white blood cells.
· Transcription factor – a protein that either up- or down- regulates the copying of RNA (transcription) from DNA. They often have domains that attach to specific sequences of DNA nucleotides. Some attach to other proteins that attach to the DNA. Or both. Up-regulate- turn on pathways (sometimes related to positive feedback).
· Upstream- the end of the DNA or RNA with the free 5' carbon of the (deoxy) ribose. Nucleic acid synthesis and translation proceeds 5' to 3'.
· Zymosan – cell wall wall material of fungi
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