Administrative issues:

Recommended text: Goldsby/Kuby Immunology, 6th edition  
(Note that Innate Immunity is not adequately covered in the 5th edition.)

Text book reading assignments are to supplement the lecture. Exam questions will be drawn primarily from lecture material.

Discussion sections start next week. The journal article Akira et al, and the relevant problem set questions will be covered. Both are available on the website.

Office Hours: Questions about the lecture material are best addressed during office hours (Tues 11-12). I will be holding extra office hours (date and time TBA) before the first midterm.

Email: Please use email only for VERY simple yes/no questions or simple administrative matters.

Great questions, keep them coming!

Antigens & Antibodies I

Discovery of antibodies

Basic Antibody Structure

brief review of protein structure

disulfide linked tetramer: 2 heavy and 2 light chains

myeloma proteins, Ig domains, and hypervariable regions

The antigen binding site of antibodies

Antibody isotypes: IgM, IgG, IgD, IgA, IgE

The advantages of multivalency

effecter functions of antibody isotypes

Myeloma protein: key to determining Ig structure

• Heterogeneity of antibodies makes sequencing impossible (each B cell clone produces a unique version of antibody).
• Multiple myeloma: cancer derived from an antibody producing cells (plasma B cell).
• Myeloma patients have large amounts of one particular Ig molecule in their serum (and urine).
• Many patients produce a large amount of one light chain, known as “Bence-Jones” proteins.

Protein homology

• Identity or similarity between domains in two or more proteins
• Most easy to see at the level of primary amino acid sequence (computer programs find it)
• Sometimes no obvious primary sequence homology but striking structural homology
• Homology can sometimes predict structure and function
All Ig domains have a similar 3D structure known as an “Immunoglobulin Fold”.

2 β-pleated sheets come together to form a sandwich, held together by disulfide bond and hydrophobic interactions.

3 flexible loops at end: correspond to hypervariable regions of primary sequence (HV).

The Immunoglobulin Fold is a very commonly used structural motif amongst cell surface proteins.

The variability of antibodies occurs within 3 discrete regions of the primary sequence: hypervariable regions HV1-3.

The quaternary structure of immunoglobulin

6 CDR (3 from HC, 3 from LC) combine to make up antigen binding site.

Complementary Determining Regions or CDR1-3.

The hypervariable regions (HV1-3) are separated in primary structure, but come together in the tertiary structure where they form the antigen binding site.

The HV regions form loops at the end of the Ig domain.

The intervening framework regions (FR1-4) make up the rest of the structure.

Ig domain: Genome Project Champion!
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The advantages of multivalency
effector functions of antibody isotypes

Antigen-antibody interactions regions come in many shapes including: pockets, grooves, or extended flat surfaces.

Because the CDR are highly variable, each antibody molecule has a unique antigen binding site with its own dimensions and complementarity.

Antigen

Epitope - three-dimensional face of an antigen which makes contact with the antibody
"Conformational Epitopes"

denatured

Native structure

B cell epitopes can be sequential (linear) or non sequential (conformational)

Five sequential epitopes in whale myoglobin

A non-sequential epitope in hen lysozyme

Demonstration of the importance of conformation in antibody-antigen binding.

Epitope and antigen binding site form complementary surfaces

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crystal structure of antibody: the Ig domain

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Differences in valency and tissue distribution

effector functions of antibody isotypes

Heavy and light chains come in different types
Ig isotypes are due to differences in heavy-chain or light-chain constant region sequences.

Heavy chains come in 5 major types that have different tissue distributions and effector functions: \( \gamma, \mu, \delta, \alpha, \varepsilon \)

Light chains come in two major types: \( \kappa \) or \( \lambda \).

Antibodies protect by recruiting other effector functions through the interaction of \( \text{C}_\text{H} \) domains with other cells and proteins of the immune system.

Different antibody isotypes recruit different effector functions.

Receptors that bind to the Fc portion of antibodies are called “Fc receptors”.

Multivalency leads to tighter binding.

<table>
<thead>
<tr>
<th>Class</th>
<th>Heavy chain</th>
<th>Subclasses</th>
<th>Light chain</th>
<th>Molecular formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG</td>
<td>( \gamma )</td>
<td>( \gamma_1, \gamma_2, \gamma_3, \gamma_4 )</td>
<td>( \kappa ) or ( \lambda )</td>
<td>( \gamma_\kappa ) or ( \gamma_\lambda )</td>
</tr>
<tr>
<td>IgM</td>
<td>( \mu )</td>
<td>None</td>
<td>( \kappa ) or ( \lambda )</td>
<td>( \mu_\kappa ), ( \mu_\lambda )</td>
</tr>
<tr>
<td>IgA</td>
<td>( \alpha )</td>
<td>( \alpha_1, \alpha_2 )</td>
<td>( \kappa ) or ( \lambda )</td>
<td>( \alpha_\kappa ), ( \alpha_\lambda )</td>
</tr>
<tr>
<td>IgE</td>
<td>( \epsilon )</td>
<td>None</td>
<td>( \kappa ) or ( \lambda )</td>
<td>( \epsilon_\kappa ), ( \epsilon_\lambda )</td>
</tr>
<tr>
<td>IgD</td>
<td>( \delta )</td>
<td>None</td>
<td>( \kappa ) or ( \lambda )</td>
<td>( \delta_\kappa ), ( \delta_\lambda )</td>
</tr>
</tbody>
</table>

Advantage of multivalency

Decameric IgM

Dimeric IgG
What are the virtues of valence?

10 antigen binding Sites/molecule

4 antigen binding Sites/molecule

Some classes of Immunoglobulin (IgG, IgD and IgA) have a flexible, proline-rich hinge region. Flexibility of antibody arms allow for more efficient binding to multivalent antigens.

Antibody “arms” are connected by a flexible hinge

Distribution of various Ig isotypes in body fluids

Relative amount Ig

IgM | IgG | IgA | IgE

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differences in valency and tissue distribution

effector functions of antibody isotypes
Neutralization

• Neutralization: binding itself prevents pathogenesis.
• Opsonization: enhancing phagocytosis
• Complement activation

Antibody-Dependent Cellular Toxicity (ADCC)

IgM

• pentameric (decavalent)
• Pentameric structure held together by J-chain and disulfide bonds.
• First Ig produced in response to infection
• Good at complement activation
IgA

- dimeric (tetravalent) predominant Ig in secretions.
- Transported across epithelial cells via poly-Ig receptor.
- 10g of IgA secreted/day, more than any other Ig!
- Found in breast milk, supplies passive immunity to baby.

IgE

- Present in VERY LOW amounts in serum
- Binds to Fc Receptors present on mast cells and basophils
- Levels increase in setting of parasitic infection
- Can transfer allergy between individuals
Antigens & Antibodies II

Definitions
A comparison of antigen recognition by B and T cells
Factors that determine immunogenicity
Quantitating the strength of antibody-antigen interactions: affinity and avidity
Impact of multivalency
Cross-reactivity of antibodies
Measuring antibody-antigen binding

The antigen receptor of B cells (antibody) binds directly to antigen. Antibody exists in both a transmembrane receptor and secreted form.

The antigen receptor of T cells (TCR) binds processed antigen (peptide) on the surface of an antigen-presenting cell. TCR exists only as transmembrane form.

Definitions
- **Antibody**: a protein (immunoglobulin) that binds an antigen.
- **Antigen**: a substance that is recognized by the immune system
- **Immunogen**: a substance that elicits an immune response (not all antigens are immunogenic!)
- **Epitope**: the portion of an antigen that is recognized by the antibody (or TCR). Also called "antigenic determinant"
- **Hapten**: a small molecule that cannot by itself induce an immune response, but can be an antigen.

**Table 9.2: Comparison of antigen recognition by T cells and B cells**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>B cells</th>
<th>T cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interaction with antigen</td>
<td>回忆性二聚体与抗原</td>
<td>回忆性T细胞与抗原</td>
</tr>
<tr>
<td>Binding of soluble antigen</td>
<td>是</td>
<td>否</td>
</tr>
<tr>
<td>Requirement for MHC molecules</td>
<td>是</td>
<td>是</td>
</tr>
<tr>
<td>Chemical nature of antigen</td>
<td>蛋白,多糖,脂质</td>
<td>蛋白,多糖,脂质</td>
</tr>
<tr>
<td>Epitope properties</td>
<td>可识别多糖,脂质,蛋白质</td>
<td>可识别多糖,脂质,蛋白质</td>
</tr>
</tbody>
</table>

Note: Table 9.2 is adapted from Chapter 9. Source: Antigens & Antibodies II, by M. A. Carbone and Company.
Immunogenicity

- **Foreignness** -- greater difference from host
- **Size** -- bigger is better
- **Complexity** -- polyglycine is a poor immunogen
- **Susceptibility to phagocytosis** -- particles better than soluble material
- **Genotype of host** -- esp MHC types
- **Route of administration** -- subcu better than IV
- **Dose** -- not too high, not too low

### Hapten

Hapten: a small molecule that cannot by itself induce an immune response, but can be an antigen.

![Hapten Diagram]

Even closely related haptens can be distinguished antigenically; antibodies raised against 1,2 DNP may not react with 1,3 DNP.

Adjuvants enhance the immunogenicity of antigens by:
- triggering the innate immune system (many contain TLR agonists)
- promoting phagocytosis of antigen, others?

<table>
<thead>
<tr>
<th>Adjuvant</th>
<th>Induced antigen presentation</th>
<th>Enhance cytokine signal</th>
<th>Improve antibody formation</th>
<th>Reduce bystander activation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Freund’s incomplete adjuvant</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Freund’s complete adjuvant</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Mycobacteria extracts</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Bacterial lipopolysaccharide</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Bacterial flagellin</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Bacterial peptidoglycan</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Bacterial ribosomal RNA</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Bacterial DNA</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

The first adjuvant Freund’s complete adjuvant: emulsified mineral oil and mycobacterial extract.

The most effective adjuvants cannot be used in humans due to toxicity (exception: diphtheria-pertussis-tetanus combined vaccine (DPT)).

### Haptens

Haptens are not immunogenic unless they are coupled to a carrier protein.

![Hapten-Carrier Diagram]

### Antigens & Antibodies II

**Definitions**
- A comparison of antigen recognition by B and T cells
- Factors that influence immunogenicity

**Quantitating the strength of antibody-antigen interactions**
- Equilibrium constants
- Equilibrium dialysis
- Impact of multivalency

**Cross-reactivity of antibodies**

**Measuring antibody-antigen binding**

### Epitope and Antigen Binding Site

Form complementary surfaces
Quantitating antibody-antigen interactions:
Strength is determined by the sum of multiple non-covalent bonds. Strength of interaction between a single epitope and antigen binding site is called its affinity. Each antibody-antigen interaction has a distinct affinity.

Measuring affinity by equilibrium dialysis
Once you know the concentration of free and bound ligand at equilibrium for different ligand concentrations, you can calculate the equilibrium binding constant \( K_a \), which provides a quantitative measure of the affinity of the interaction.

Note that equilibrium dialysis is based on differential ability of ligand and antibody to pass through membrane. Can only be used when the ligand is small (e.g. a hapten).

Equilibrium binding equation

\[
Ag + Ab \xrightleftharpoons[k_2]{k_1} Ag-Ab
\]

Free
Free
Antigen-antibody complex

\[
K_a = \frac{[Ab-Ag]}{[Ab][Ag]}
\]

Ka is the association binding constant.
\( k_1 \) or \( k_{on} \) is the association rate constant.
\( k_2 \) or \( k_{off} \) is the dissociation rate contant.

If binding is weak: \( k_2 \) (off rate) is high, and \( K_a \) (association binding constant) will be low (equilibrium shifted to the left).

If binding is strong: \( k_2 \) (off rate) is low, and \( K_a \) will be high (equilibrium shifted to the right).

Equilibrium binding equation

\[
Ag + Ab \xrightleftharpoons[k_2]{k_1} Ag-Ab
\]

\[
K_a = \frac{[Ab-Ag]}{[Ab][Ag]}
\]

Sometimes binding strength is represented by \( K_d \) (dissociation equilibrium constant) = 1/\( K_a \)

\[
Ag-Ab \xrightleftharpoons[k_1]{k_2} Ag + Ab
\]

\[
K_d = \frac{[Ab][Ag]}{[Ab-Ag]}
\]

\( K_d \) (dissociation equilibrium constant) = 1/\( K_a \) (units are moles/liter)

The concentration at which 50% of the antibody and ligand are bound at equilibrium, is close to the \( K_d \)

The ligand concentration at which 1/2 of the antibody is binding ligand at equilibrium, is close to the \( K_d \)

Stronger binding corresponds to lower \( K_d \)

\[
K_d = \frac{[Ab][Ag]}{[Ab-Ag]}
\]

If the concentration of total antibody and antigen (bound and free) is 2 x 10^{-7} M (moles/liter):

For an interaction whose \( K_d = 10^{-7} \)M:

50% of antibody and antigen are bound

\[
10^{-7} M = \frac{10^{-7} M \times 10^{-7} M}{10^{-7} M}
\]

For an interaction whose \( K_d = 0.5 \times 10^{-9} \)

95% of antibody and antigen are bound

\[
0.5 \times 10^{-9} M = \frac{10^{-9} M \times 10^{-9} M}{1.9 \times 10^{-9} M}
\]
Note that for two antibody-ligand pairs with similar on rates ($k_1$), a lower off rate ($k_{-1}$) corresponds to tighter binding (higher $K_a$, lower $K_d$).

Note that for two antibody-ligand pairs with similar off rates ($k_{-1}$), a faster on rate ($k_1$) corresponds to tighter binding (higher $K_a$, lower $K_d$).

Data from equilibrium dialysis can be analyzed using Scatchard Plot:

$r = \text{bound ligand} / \text{total antibody}$

$c = \text{free ligand}$

$n = \text{number of binding sites per antibody molecule}$

Slope = $-K_a$

$\text{X-intercept} = n$

Note: this only works if the antibody is homogeneous: all antigen binding sites identical, e.g., myeloma protein or a monoclonal antibody.

What happens with polyclonal antibody which consists of mixtures of many different types of antibodies?

Polyclonal antiserum

Can be generated by repeated immunization of animal (rabbit) with antigen (with adjuvant).

Polyclonal antibodies are a complex mixture of antibodies directed against different epitopes and that differ in their affinity for the antigen.

Polyclonal antibodies vs Monoclonal antibodies

Polyclonal antibodies: antibody preparations from immunized animals. Consist of complex mixtures of different antibodies produced by many different B cell clones

Monoclonal Antibody: homogeneous antibody preparations produced in the laboratory. Consist of a single type of antigen binding site, produced by a single B cell clone (later we’ll talk about how these are made).

Affinity between two macromolecules (antibody and protein antigen) can be measured using a biosensor.

- Resonance units are proportional to the degree of binding of soluble ligand to the immobilized receptor. (or soluble antibody to immobilized antigen, as shown here)
- Determining the amount of binding at equilibrium with different known concentrations of receptor (antibody) and ligand (protein antigen) allows you to calculate equilibrium constants ($K_a, K_d$).
- Rate of dissociation and association ($k_{off}, k_{on}$) can also be calculated.
Affinity refers to strength of binding of single epitope to single antigen binding site.

But antibodies have 2 or more identical binding sites.

Most antigens are multimeric.

What is impact of valence on strength of binding?

Avidity (strength of binding) is influenced by both Affinity (Ka of single binding site) and the Valence of the interaction (number of interacting binding sites).

Decameric IgM

Dimeric IgG

low affinity interactions can have high avidity if valence is high.

IgM tend to bind tightly, but have less specificity.

Avid binding due to high affinity. Binding of IgG tends to be more specific. (more perfect "fit" between antigen binding site and antigen)