Chapter 6

Antigen-Antibody Interactions: Principles and Applications

Antigen-Antibody Properties

- You must remember antibody affinity (single) VS avidity (multiple)
- High affinity: bound tightly and longer!
- Cross-reactivity: occurs when two different antigens share an identical or a very similar epitope. The antibody’s affinity for the cross-reacting epitope will be ______ than for the original epitope.
- ABO blood groups and infectious diseases (Streptococcal M antigens, Jenner?)

Precipitin reactions

The interaction of antibody with antigen in solution may cause formation of an insoluble lattice that will precipitate out of solution.

This precipitate will only form if:
- The antibody is bivalent or polyvalent
- The antibody or antibody mixture can bind to at least two different sites on the antigen (either two different epitopes or two identical epitopes)

Monoclonal antibodies are likely to be less efficient at immunoprecipitation than polyclonal antibodies.

<p>| Table 6-3 |</p>
<table>
<thead>
<tr>
<th>Sensitivity of various immunomaps</th>
<th>Limits (ng antibody/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Precipitation reactions</td>
<td>20 - 1,000</td>
</tr>
<tr>
<td>Mouse radial immunodiffusion</td>
<td>20 - 1,000</td>
</tr>
<tr>
<td>Coomassie-Blue double immunodiffusion</td>
<td>20 - 1,000</td>
</tr>
<tr>
<td>Immunoelectrophoresis</td>
<td>20 - 1,000</td>
</tr>
<tr>
<td>Rabbit immunoelectrophoresis</td>
<td>20 - 1,000</td>
</tr>
<tr>
<td>Flow immunofluorescence</td>
<td>0.2 - 20</td>
</tr>
<tr>
<td>ELISA using the antigen</td>
<td>0.2 - 20</td>
</tr>
<tr>
<td>Immunofluorescence</td>
<td>0.2 - 20</td>
</tr>
</tbody>
</table>

*For sensitivity depends on the affinity of the antibody used for the assay as well as the antigen density and distribution on the antigen.

Table 6-3 Sensitivity of various immunomaps (Kuby 4th Edition)
Double Immunodiffusion (Ouchterlony)

Diffusion of antibody and antigen towards each other in an agarose gel.
A line of precipitate will form if the antibody binds to antigen.
Used to determine if an antigen or antibody is present.

Radial Immunodiffusion

Diffusion of antigen through an agarose gel containing antibody.
A precipitin ring will form. The size of the ring is proportional to the antigen concentration.
Used to determine concentrations of specific proteins (low sensitivity)

Hemagglutination

Antibody can also cross-link cells or beads.
Cross-linking of red cells is called hemagglutination.
Non-cross-linked cells settle in a bead to the bottom of the well.
Cross-linked cells settle in a diffuse pattern.
Used to measure antibody presence and level (titer).
Used to measure antibodies to red cell antigens or to other antigens bound to the surface of red cells.
**Immunoelectrophoresis**

1. Antigens are electrophoresed to separate them based on their charge.
2. A trough is cut and antiserum is added.

**Enzyme-linked immunosorbent assay (ELISA)**

Used to measure antigen or antibody presence and concentration.

- Far more sensitive than precipitin or agglutination techniques.
- Relies on the ability to covalently conjugate chemicals to the Fc region of Ig without interfering with antigen binding ("enzyme-linked") and the ability of plastic to nonspecifically bind proteins (immunosorbent).
- In ELISA, an enzyme is bound to the Fc region - usually horseradish peroxidase or alkaline phosphatase. Enzyme presence can be determined by use of colorimetric substrates.
- Final measurement is an absorbance.
- Comparison with standard curves indicates concentration of antigen or antibody.
- Various assay formats are possible.

**Sample Absorbance**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive control</td>
<td>1.689</td>
</tr>
<tr>
<td>Negative control</td>
<td>0.153</td>
</tr>
<tr>
<td>Assay control</td>
<td>0.123</td>
</tr>
<tr>
<td>Patient A</td>
<td>0.055</td>
</tr>
<tr>
<td>Patient B</td>
<td>0.412</td>
</tr>
<tr>
<td>Patient C</td>
<td>1.999</td>
</tr>
</tbody>
</table>
**Relative sensitivities:**

Precipitin reactions < Agglutination reactions < ELISA

**Western blotting** - Used to identify presence of a specific antigen or antibody

1) Proteins (Ag) are separated by polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a nitrocellulose-style sheet (or other).

2) Sheet is incubated with anti-Ag antibody

3) Band appears wherever the Ag is present.

**HIV Western Blot**

No bands present..........................Negative

Bands at either p31 OR p24 AND bands present at either gp160 OR gp120.............Positive

Bands present, but pattern does not Meet criteria for positivity................................Indeterminate

1. Lane 1, HIV+ serum (positive control)
2. Lane 2, HIV- serum (negative control)
3. Lane A, Patient A
4. Lane B, Patient B
5. Lane C, Patient C

http://www.biology.arizona.edu/immunology/activities/western_blot/west2.html
**Immuno-magnetic separation (IMAS)**

- Technique used to separate cells, proteins, nucleic acids using antibodies or ligands-bound to magnetic beads.
- Removed out from mix suspension using a magnet.
Fluorescence Activated Cell Sorter (FACS)

- Mixed cell population
- Requires two different fluorochromes
- Commonly used: FITC, PE, Texas Red, etc.

ALL of the T lineage

- CD4 (coreceptor for MHC II)
- CD8 (coreceptor for MHC I)
- CD1 (an MHC class I-like molecule)
- CD5 (a T-cell marker)
- CD2 (an adhesion molecule)
- CD7 (marker of some T cells, thymocytes and pluripotent hematopoietic cells)

A B-lineage CLL

- Ig (low-affinity IgE receptor)
- CD23 (adhesion molecule)
- CD19
- CD20 (B-cell marker)
- CD34
- CD44 (adhesion molecule)
- MHC II

SSC – SIDE SCATTERED LIGHT proportional to granularity or internal complexity

FSC – FORWARD SCATTERED LIGHT proportional to size or surface area

FACS Analysis

Reagents:
- CD4-PE Phycoerythrin (PE)
- CD3 Fluorescein (FITC)
Markers for Th cells

- Naïve – CD62L (L-selectin), CD45RA
- Activated – CD25, CD69, CD38
- Memory – CD45RO, CD44

High (hi) vs Low (lo)

The Alexa Fluor Dye Series

<table>
<thead>
<tr>
<th>Probe</th>
<th>Ex (nm)</th>
<th>Em (nm)</th>
<th>MW</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cascade Blue</td>
<td>425</td>
<td>423</td>
<td>596</td>
</tr>
<tr>
<td>Lucifer yellow</td>
<td>425</td>
<td>528</td>
<td></td>
</tr>
<tr>
<td>Phycocerythrin (PE)</td>
<td>480-565</td>
<td>578</td>
<td>240 k</td>
</tr>
<tr>
<td>Fluorescein (FITC)</td>
<td>495</td>
<td>519</td>
<td>389</td>
</tr>
<tr>
<td>BODIPY-FL</td>
<td>503</td>
<td>512</td>
<td></td>
</tr>
<tr>
<td>TRITC</td>
<td>547</td>
<td>572</td>
<td>444</td>
</tr>
<tr>
<td>Rhodamine</td>
<td>570</td>
<td>576</td>
<td>548</td>
</tr>
<tr>
<td>Texas Red</td>
<td>589</td>
<td>615</td>
<td>625</td>
</tr>
<tr>
<td>TruRed</td>
<td>490/675</td>
<td>695</td>
<td></td>
</tr>
</tbody>
</table>