

Chapter 6: Antigen-Antibody Interactions

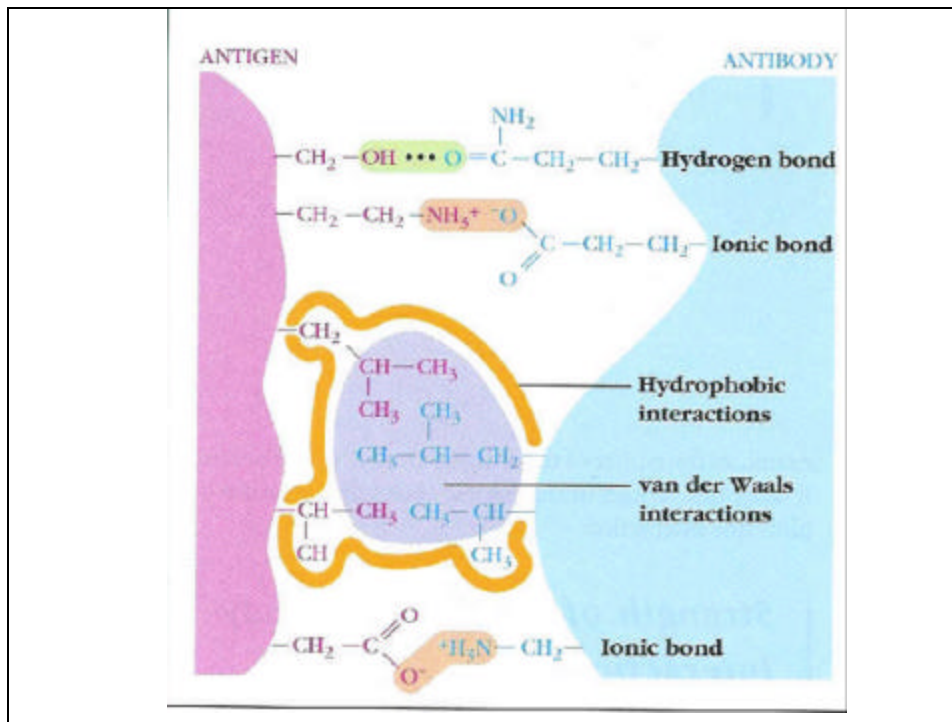
- I. Strength of Ag-Ab interactions
 - A. Antibody Affinity
 - strength of total noncovalent interactions between single Ag-binding site on an Ab and a single epitope is the affinity of the Ab for that epitope
 - low affinity Ab: bind Ag weakly and dissociate readily
 - high affinity Ab: bind Ag tightly and remain bound longer

- K_a (measure of Ab affinity) can be calculated from the ratio of the molar concentration of bound Ag-Ab complex to the molar concentrations of unbound Ag and Ab at equilibrium

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

- rate at which bound Ag leaves the Ab's binding site helps determine the Ab's affinity for an Ag

- noncovalent forces involved in Ag-Ab interaction: hydrogen bonds, ionic bonds, hydrophobic interactions, and van der Waals forces



B. Antibody Avidity

- strength of multiple interactions between a multivalent Ab and Ag
- avidity of an Ab is a better measure of its binding capacity within biological systems than the affinity of its individual binding sites
- high avidity can compensate for low affinity

II. Cross-Reactivity

- Ab elicited by one Ag can cross-react with an unrelated Ag
- cross-reactivity occurs if 2 different Ag's share an identical epitope or if Ab's specific for one epitope also bind to an unrelated epitope possessing similar chemical properties

- example of cross-reactivity:
 - ABO blood-group antigens
 - subtle differences in the terminal sugar residues distinguish the A and B blood-group antigens
 - cross-reactivity is the basis for the presence of these blood-group antibodies, which are induced in an individual not by exposure to red blood cell antigens but by exposure to cross-reacting microbial antigens present on common intestinal bacteria

TABLE 6-2 ABO BLOOD TYPES

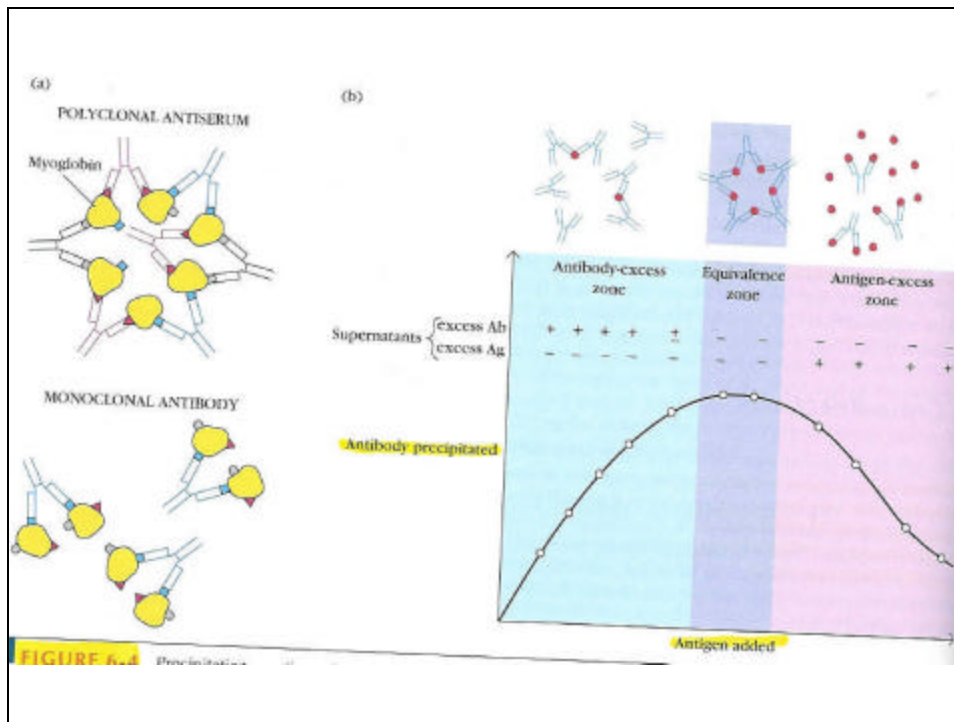
Blood type	Antigens on RBCs	Serum antibodies
A	A	Anti-B
B	B	Anti-A
AB	A and B	Neither
O	Neither	Anti-A and anti-B

III. Precipitation Reactions

- antibodies that aggregate soluble antigens are called precipitins
- formation of an Ag-Ab lattice depends on the valency of both the Ab and Ag:
 - the Ag must be bivalent; a precipitate will not form with monovalent Fab fragments
 - the Ag must be either bivalent or polyvalent; that is, it must have at least two copies of the same epitope, or have different epitopes that react with different antibodies present in polyclonal antisera

A. Precipitation Reactions in Fluids

- excess of either Ab or Ag interferes with maximal precipitation, which occurs in the equivalence zone, when the ratio of Ab to Ag is optimal
 - in the region of Ab excess, unreacted Ab is found in the supernatant along with small, soluble complexes consisting of multiple molecules of Ab bound to a single molecule of Ag
 - in the region of Ag excess, unreacted Ag can be detected and small, soluble complexes are again observed, this time consisting of 1 or 2 molecules of Ag bound to a single molecule of Ab



B. Precipitation Reactions in Gels

- immunodiffusion reactions can be used to determine relative concentrations of Abs' or Ags', to compare Ags', or to determine the relative purity of an Ag preparation

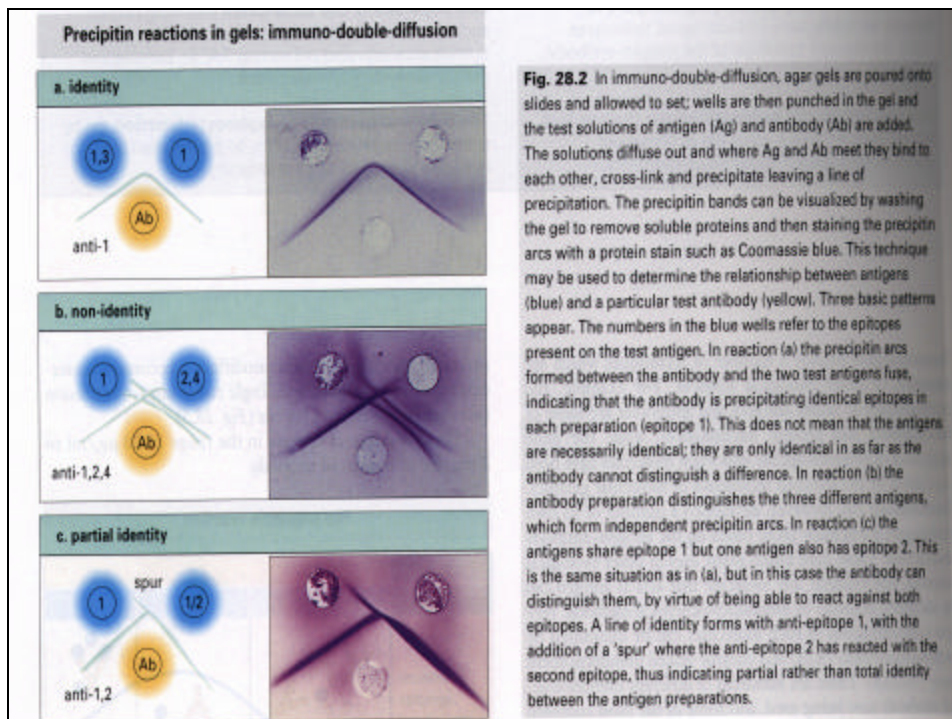
1. Radial Immunodiffusion

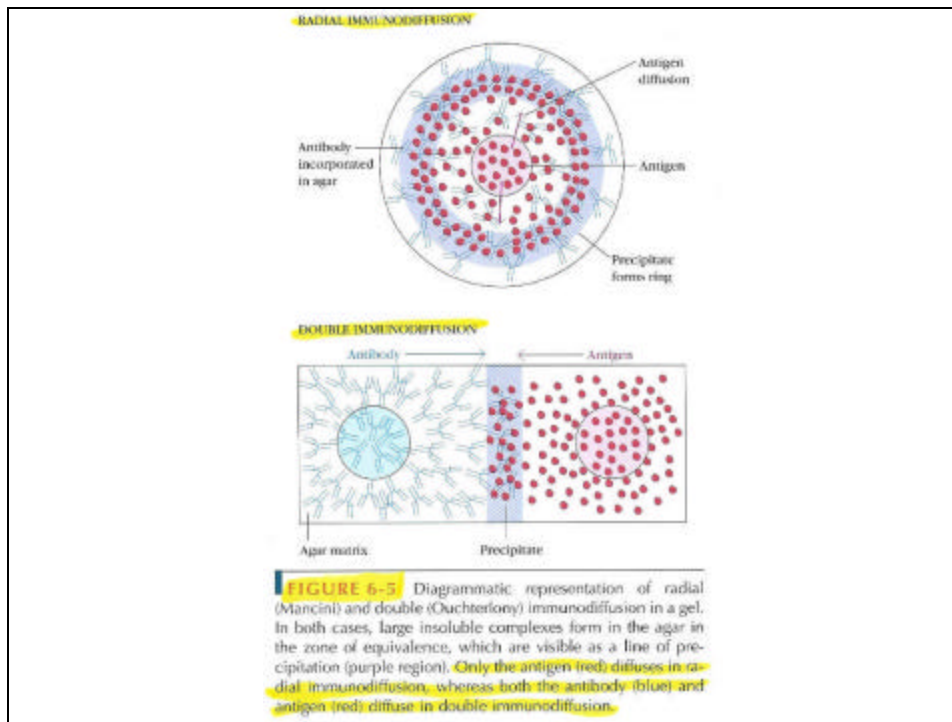
- Ag sample is placed in a well and allowed to diffuse into agar containing a suitable dilution of an antiserum

- area of precipitin ring formed is proportional to the concentration of Ag

2. Double Immunodiffusion

- qualitative tool for determining the relationship between antigens and the number of different Ag-Ab systems present
 - identity: occurs when two antigens share identical epitopes
 - nonidentity: occurs when two antigens are unrelated (share no common epitopes)
 - partial identity: occurs when two antigens share some epitopes but one or the other has a unique epitope





3. Immunoelectrophoresis

- combines separation by electrophoresis with identification by double immunodiffusion

- useful in determining whether a patient produces abnormally low amounts of one or more isotypes, characteristic of certain immunodeficiency diseases

IV. Agglutination Reactions

- interaction between Ab and a particulate Ag results in clumping called agglutination
- Ab excess can inhibit agglutination reactions, which is called prozone effect

- at high [Ab], the number of Ab binding sites may greatly exceed the number of epitopes, which results in antibodies being able to bind antigen only univalently instead of multivalently

- Ab's that bind univalently cannot crosslink one Ag to another

A. Hemagglutination

- agglutination reactions that are performed on RBC's

- RBC's mixed with antisera to the A or B blood-group antigens on a slide

- if antigen is present on the cells, they agglutinate, forming a visible clump on slide

B. Bacterial Agglutination

- bacterial infection often elicits the production of serum antibodies specific for surface antigens on the bacterial cells

- presence of such antibodies can be detected by bacterial agglutination reactions

- serum antibody titer of a patient is defined as the reciprocal of the greatest serum dilution that elicits a positive agglutination reaction

- agglutination titer of an antiserum can be used to diagnose a bacterial infection

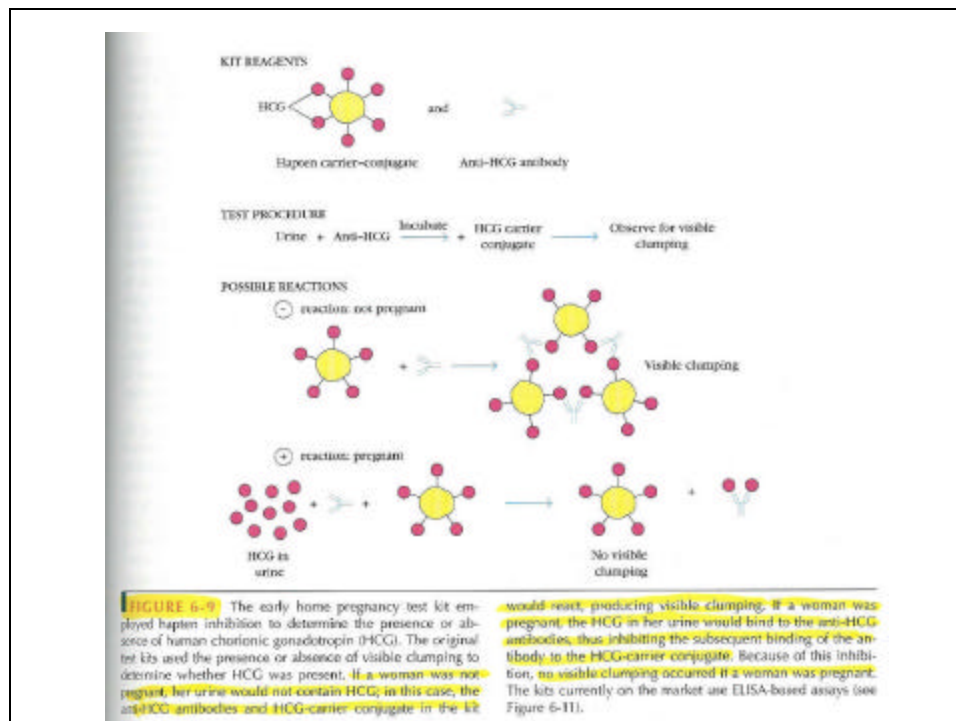
C. Passive Agglutination

- the sensitivity and simplicity of agglutination reactions can be extended to soluble antigens by the technique of passive hemagglutination

D. Agglutination Inhibition

- provides a highly sensitive assay for small quantities of an Ag

- ex. First home pregnancy test



V. Radioimmunoassay

- one of the most sensitive techniques for detecting antigen or antibody
- principle involves competitive binding of radiolabeled Ag and unlabeled Ag to a high affinity Ab
- ratio of Ab to radioactive Ag is chosen such that the number of epitopes presented by the labeled Ag always exceeds the total number of Ab binding sites
- this insures that any unlabeled Ag added to the sample mixture will compete with radiolabeled Ag for the limited supply of Ab

VI. Enzyme-Linked Immunosorbent Assay

- also known as ELISA
- an enzyme conjugated with an Ab reacts with a colorless substrate to generate a colored reaction product
 - substrate known as chromogenic substrate

A. Indirect ELISA

- Ab can be detected or quantitatively determined
- method of choice to detect the presence of serum antibodies against HIV

B. Sandwich Assay

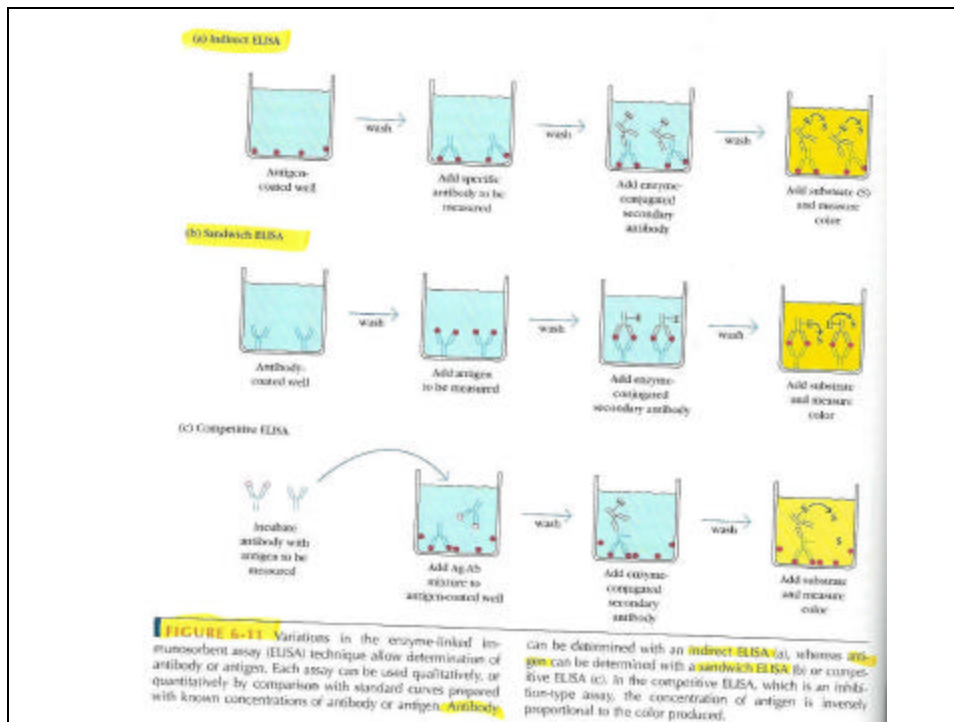
- Ab (rather than Ag) is immobilized on a microtiter well
- sample containing Ag is added and allowed to react with immobilized Ab
- After well is washed, a second enzyme-linked Ab specific for a different epitope on the Ag is added and allowed to react with the bound Ag
- after any free 2nd Ab is removed by washing, substrate is added, and the colored reaction product is measured

C. Competitive ELISA

- Ab is first incubated in solution with a sample containing Ag
- Ag-Ab mixture is then added to an Ag-coated microtiter well
- addition of an enzyme-conjugated secondary Ab specific for the isotype of the primary Ab can be used to determine the amount of primary Ab bound to the well as in an indirect ELISA

D. Chemiluminescence

- a luxogenic (light-generating) substrate takes the place of the chromogenic substrate in conventional ELISA reactions



E. ELISPOT Assay

- allows the quantitative determination of the number of cells in a population that are producing antibodies specific for a given Ag or an Ag for which one has a specific Ab

VII. Western Blotting

- identification of a specific protein in a complex mixture of proteins
- a protein mixture is electrophoretically separated by SDS-PAGE
- proteins visualized by:
 - use of radioactive Ab
 - enzyme-linked Ab against protein
- can also identify a specific Ab in a mixture
 - used for confirmatory testing for HIV

VIII. Immunoprecipitation

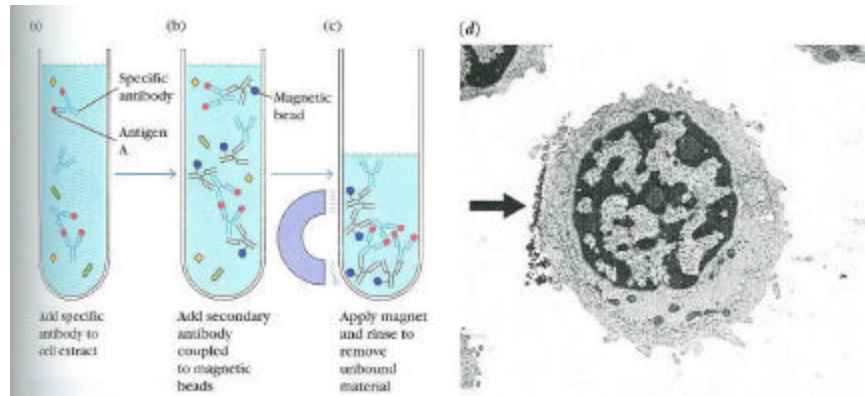


FIGURE 6-14 Immunoprecipitates can be collected using magnetic beads coupled to a secondary antibody. (a) Treatment of a cell extract containing antigen A (red) with a mouse anti-A antibody (blue) results in the formation of antigen-antibody complexes. (b) Addition of magnetic beads to which a rabbit anti-mouse antibody is linked binds the antigen-antibody complex (and any unreacted mouse Ig). (c) Placing a magnet against the side of the tube allows the rapid collection of the antigen-antibody complexes. After rinsing to remove any unbound material, the antigen-antibody complexes can be dissociated and the antigen studied. (d) An electron micrograph showing a cell with magnetic beads (arrow) attached to its surface via antibodies. [Part (d) P Groscurth, Institute of Anatomy, University of Zurich-Itchel.]

IX. Immunofluorescence

- most commonly used fluorescent dyes are fluorescein and rhodamine, but other highly fluorescent substances (phycoerythrin and phycobiliproteins) have also come into use
 - fluorescein: absorbs blue light and emits an intense yellow-green fluorescence
 - rhodamine: absorbs yellow-green range and emits a deep red fluorescence
 - phycoerythrin: efficient absorbers of light and brilliant emitters of red fluorescence

X. Flow Cytometry and Fluorescence

- FACS (fluorescence-activated cell sorter) was designed to automate the analysis and separation of cells stained with fluorescent Ab
- FACS used to determine the kind and number of WBC's in each population in patients' blood samples
- FACS is one of the essential tools for the detection and classification of leukemias

XI. Immunoelectron Microscopy

- used to visualize specific intracellular tissue components by using the specificity of antibodies