Day 14 (Immunology)

Immunogenicity vs. Antigenicity

- Immunogenicity: ability to induce humoral or cell-mediated immune response
- Antigenicity: ability to combine specifi the final products of antibo & cell surface receptors

All molecules with immunogenicity also have antigenicity, but the reverse is not true.

- haptens are antigenic but cannot induce an immune response (not immunogenic)

Haptens

- by itself cannot function as an immunogenic epitope
- simple organic molecules — phenyl arsonates & nitrophenyls
- chemical coupling of hapten to protein carrier → immunogenic hapten-carrier conjugate

Once a hapten-carrier conjugate is formed, immunogenic antibodies includes:

1. hapten specific (major)
2. unaltered epitopes on carrier protein sp. (minor)
3. New epitopes on conjugate specific (minor)

As diagnostic tools, haptens help to probe the effect of minor variations on immunospecificity.

Landsteiner's experiment

rabbits immunised with hapten–carrier conjugate

↓

activity of rabbit's sera with that hapten and closely-related haptens with a different carrier

↓

allowed to measure reaction of antihapten antibodies without the activity specific to original carrier epitopes

↓

Testing whether anti-hapten antibodies could bind to haptens with similar structure (cross reaction)

↓

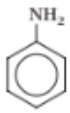
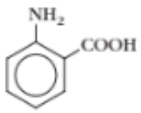
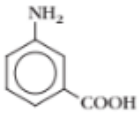
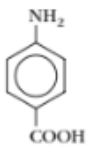
Insight into specificity of antigen antibody rxns.

Result: Overall configuration of hapten is important.

Various derivatives of aminobenzene was used as hapte

↓

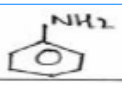
TABLE 3-6 Reactivity of antisera with various haptens

	REACTIVITY WITH			
				
Antiserum against	Aminobenzene (aniline)	*o*-Aminobenzoic acid	*m*-Aminobenzoic acid	*p*-Aminobenzoic acid
Aminobenzene	+	0	0	0
o-Aminobenzoic acid	0	+	0	0
m-Aminobenzoic acid	0	0	+	0
p-Aminobenzoic acid	0	0	0	+

However, if the overall configuration was kept the same, and hapten was modified in para position with non-ionic derivatives,

various degrees of cross-reactivity

Fig-2

Antiserum against	 Amino benzene	 P-chloro amino benzene	 P- Toluidine
Amino benzene	+/++	+	±
P-chloro amino benzene	+++	++	++
P-Toluidine	++/+	++	++
P-nitro amino benzene	+	++	+/++

Drug Allergies

- drugs function as haptens
- depending on the following factors, they can become immunogens themselves

Foreignness

- in order to elicit an immune response, body must recognise it as <u>non-self</u>

- body must also be tolerant of self-antigens (develops during lymphocyte dev)
- immunogenic response to an antigen depends on its degree of foreigness

↓

Greater the phylogenetic distance b/w two sp., greater str. and antigenic disparity

e.g., BSA is not immunogenic to a cow but is in rabbits

- some macromolecules like collagen & cytochrome c are conserved throughout → very little immunogenicity
- some components like sperm are sequestered and could act as immunogen if injected

Molecular Size

- correlation b/w molecular mass and immunogenicity
- most active → >100,000 Daltons
- molecular mass < 5000 - 10,000 Daltons → poor immunogen
- <1000 Da → sometimes immunogenic

Chemical composition

- heteropolymers more immunogenic than homopolymers
- chemical complexity contributes to immunogenicity

- four levels of organisation of protein str → also contribute to immunogenicity

Susceptibility to antigen processing

- more immunogenic big molecules are often phagocytosed and processed by T cells

↓

cannot be degraded and presented with MHC molecules

↓

poor immunogens

e.g., only L-amino acid polymers can be degraded,
D-amino acid polymers are poor antigens.

Adjuvants

- substances when mixed with an antigen and injected with it — enhance the immunogenicity of the antigen
- often used to elevate the immune response for antigen with low immunogenicity (or small amounts of antigen)

How do adjuvants enhance immunogenicity?

- convert soluble antigen proteins into particulate material (readily ingested by antigen presenting cells)
- ligands for Toll-like receptors on dendritic cells &

macrophages — stimulate innate immune responses

- contain bacteria or bacterial products — may signal macrophages or dendritic cells to become more effective antigen-presenting cells

Effects of adjuvants

1. Prolonged antigen persistence
2. Enhanced costimulatory signals
3. Increased local inflammation
4. Non-specific proliferation of lymphocytes

Adjuvants that enhance immune responses

Adjuvant name	Composition	Mechanism of action
Incomplete Freund's adjuvant	Oil-in-water emulsion	Delayed release of antigen; enhanced uptake by macrophages
Complete Freund's adjuvant	Oil-in-water emulsion with dead mycobacteria	Delayed release of antigen; enhanced uptake by macrophages; induction of co-stimulators in macrophages
Freund's adjuvant with MDP	Oil-in-water emulsion with muramyldipeptide (MDP), a constituent of mycobacteria	Similar to complete Freund's adjuvant
Alum (aluminum hydroxide)	Aluminum hydroxide gel	Delayed release of antigen; enhanced macrophage uptake
Alum plus *Bordetella pertussis*	Aluminum hydroxide gel with killed *B. pertussis*	Delayed release of antigen; enhanced uptake by macrophages; induction of co-stimulators

Antigen-antibody Interactions

- non-covalent antigen-antibody binding → hydrogen bonds
  → ionic bonds
  → hydrophobic int.
  → van der Waals
  → individually weak, hence large no. reqd. to form strong Ag-Ab interact
- each interaction operates over a very short distance of 1Å ⇒ strong Ag-Ab interaction requires high degree of complementarity b/w Ag & Ab.

Antibody affinity

- affinity – combined strength of non-covalent interactions b/w single epitope and paratope of antibody

$$Ag + Ab \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} Ag\text{-}Ab \qquad K_a = \frac{[Ag\text{-}Ab]}{[Ab][Ag]}$$

$\frac{k_1}{k_{-1}}$ = association constant K_a → measure of affinity

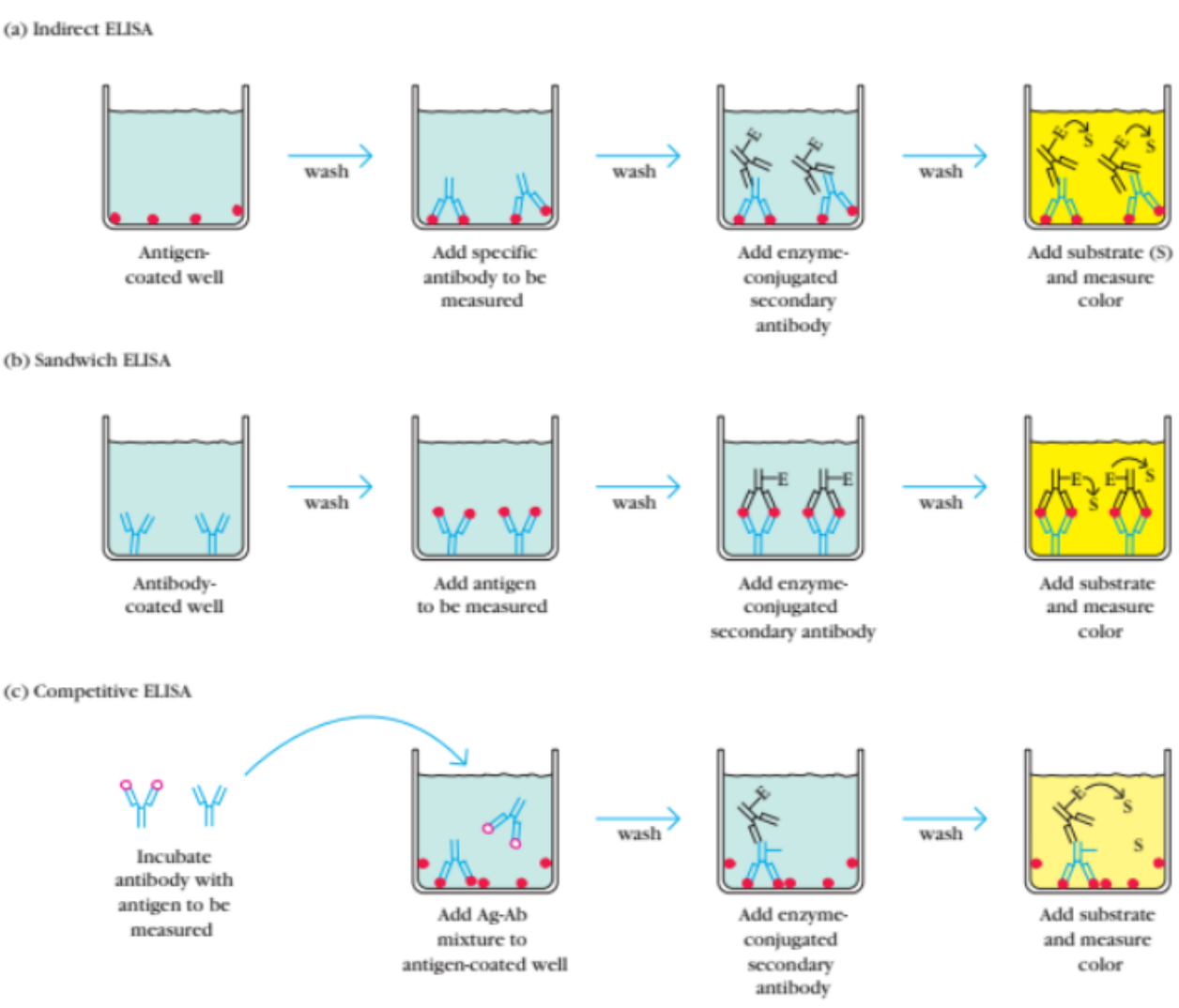
- rate at which bound antigen leaves paratope → determines affinity. ($K_d = \frac{1}{K_a}$)
- very stable complex → low values of K_d

ELISA (Enzyme-linked immunosorbent assay)

- presence of Ab to particular Ag in serum of patient can be detected
- (Enzyme + antibody) + colourless substrate → chromogenic substrate

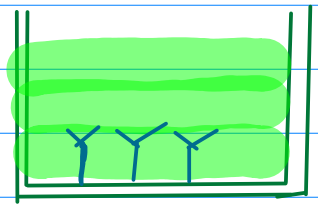
Enzymes include:
alkaline phosphatase, horseradish peroxidase, b-galactosidase

Variants of ELISA



ELISPOT Assay

- allows the quantitative determination of no. of cells that are producing antibodies specific for a given antigen

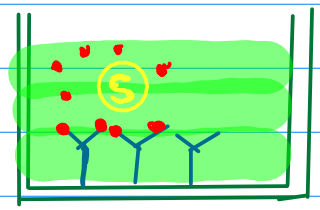


Well coated with anticytokine antibody

Add test cells

Ⓢ Secretor

Ⓝ Nonsecretor

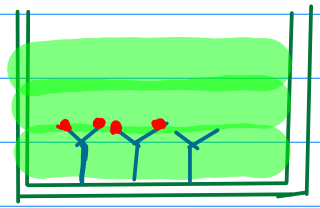


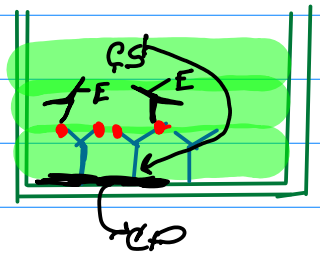
Incubate at 37°C

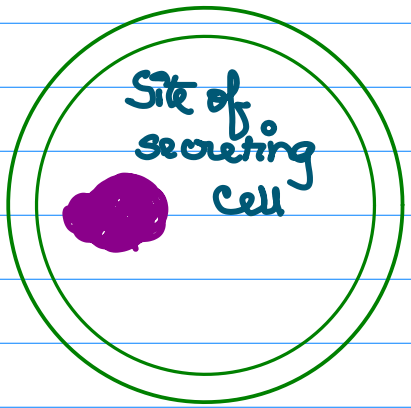
Discard cells & wash plate

E - enzyme linked
CS - Chromogenic substrate
CP - Coloured product

Enzyme linked anticytokine antibody

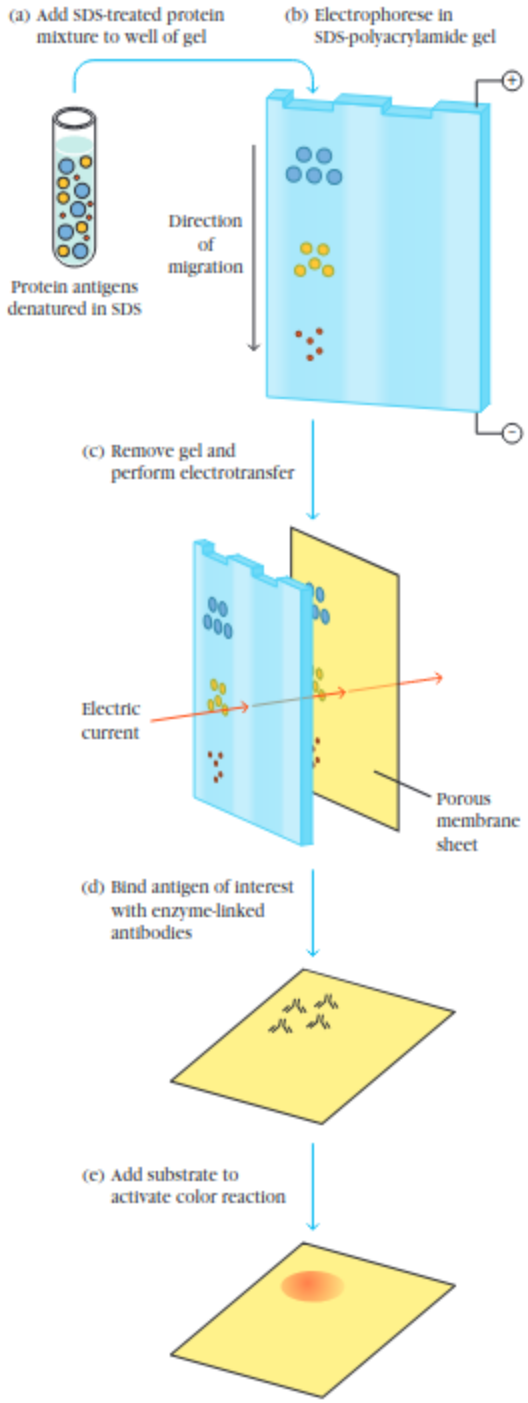






Western Blote

- identification of a specific protein in a complex mixture of proteins



Visualisation

- Enzyme linked antibody
- Autoradiography

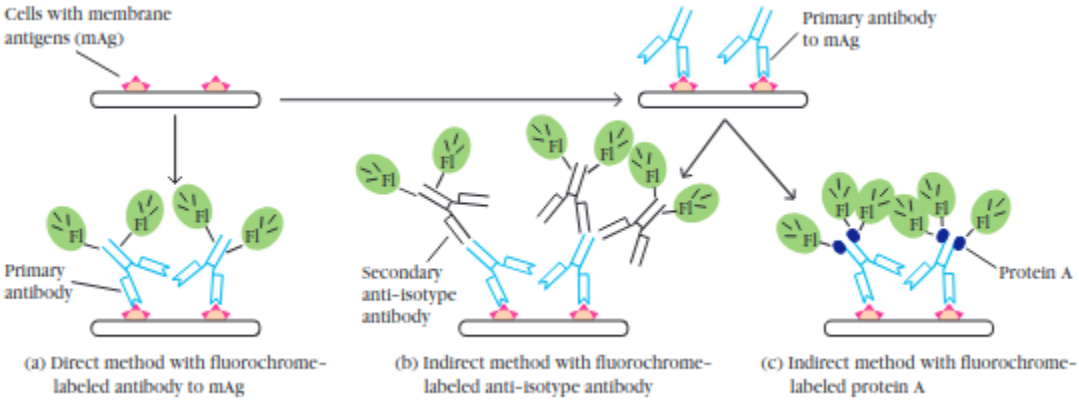
Used To Test HIV:

whether patient has antibodies that react with one or more viral proteins

SDS-dissociating agents

Immunofluorescence

- used to detect antibody molecules bound to antigens in cells
- Indirect & direct immunofluorescence



(a) Direct method with fluorochrome-labeled antibody to mAg

(b) Indirect method with fluorochrome-labeled anti-isotype antibody

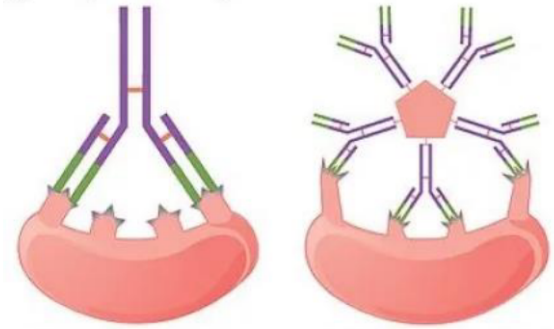
(c) Indirect method with fluorochrome-labeled protein A

Advantages of indirect immunofluorescence:

(1) primary antibody does not need to be conjugated with fluorophore — avoids loss of antibody during conjugation reaction

(2) increased sensitivity — multiple molecules of fluorochrome bind to single antibody, increasing signal intensity.

Affinity vs avidity



Affinity refers to the strength of a single antibody–antigen interaction. Each IgG antigen binding site typically has high affinity for its target.

Avidity refers to the strength of all interactions combined. IgM typically has low affinity antigen binding sites, but there are ten of them, so avidity is high.

Precipitation reactions

- antigen + antibody $\xrightarrow[\text{solution}]{\text{aqueous}}$ lattice → visible precipitate (soluble)

precipitin

Formation of Ag-Ab lattice depends on the valency of both antibody and antigen

- antibody must be bivalent – otherwise no precipitate with mono-Fab
- bivalent or polyvalent antigen – at least two copies of same epitope or different epitopes
- experiments with myoglobin → demonstrate that protein antigens must be bivalent for a precipitin rxn to occur

Myoglobin precipitates well with specific polyclonal antisera but fails to precipitate with a specific monoclonal antibody because it contains multiple, distinct epitopes but only a single copy of each epitope (Figure 6-4a). Myoglobin thus can form a crosslinked lattice structure with polyclonal antisera but not with monoclonal antisera

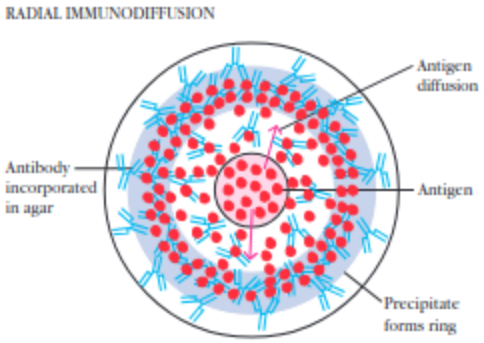
Radial immunodiffusion

- immune precipitates can form on an agar matrix
  - ↳ antigen and antibody diffuse towards each other in agar → visible line of precipitation
    - ↓ forms only in region of equivalence, not in regions of antigen and antibody excess

Uses

- To determine the relative conc. of antigen or antibody
- relative purity of antigen preparation

Methods → Radial immunodiffusion (Mancini method)



1. Antigen placed in a well in centre
2. antigen diffuses to antibody and establishes ring of precipitation
3. Area of precipitin ring ~ conc. of antigen

Agglutination reactions

- rxn b/w antibody & particulate antigen
  ↓
  visible clumping (agglutination)

antibodies producing agglutination – agglutinins

- depend on the cross linking of polyvalent antigens
- Prozone effect – excess of antibodies inhibit agglutination rxns.