Practical Applications of Immunology (Chapter 18)

Lecture Materials

for

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Primary Source for figures and content:

Applications of Immunology
1. Vaccines
2. Diagnostic tests

Vaccines
Chinese were first to “vaccinate”: variolation
-ground up small pox scabs, rub into wound or inhale
-some people got mildly ill and then were immune to small pox
-some died

Jenner developed first Western vaccine:
-observed that milk maids that got cow pox never got small pox
-he inoculated people with cow pox to prevent small pox infection
-set stage for vaccine development
(vacca = cow: name given by Pasteur later)
vaccine = suspension of organisms or fraction of organisms used to induce immunity
-only way to deal with deadly viral diseases (bacterial diseases can be treated after with antibiotics)

Mechanism of action:
-exposure (injection) induces primary immune response:
-antibodies and long term memory cells are formed
-slow, takes 1-2 weeks
-natural exposure later induces secondary immune response:
-memory cells stimulated to act
-rapid and intense response
-pathogen destroyed before it causes disease

Herd immunity = having enough of the population vaccinated to prevent spread of disease

Types of vaccines: on handout
Diagnostic Immunology
-use purified antibody solutions (antiserum) to diagnose disease
Diagnostic antibodies can be produced to detect particular microbes:
A. In animals (mixed antiserum)
   1. inject animal with microbe or antigenic fragments
   2. allow immune response (1-2 weeks)
   3. harvest blood
   4. purify antibodies from serum to make antiserum = purified antibody solution to one particular antigen
   -these preparations will produce multiple antibody types that recognize different epitopes on the antigen
B. Monoclonal antibodies
- isolate one immortalized B cell
- clone in culture
- produce cheap, pure, antiserum with one type of antibody that recognizes only one epitope on the antigen
- requires cell culture, but no need for animal husbandry and blood purification

-diagnostic immunology is the future of medical diagnosis for infectious disease: no more biochemical tests
Types of Diagnostic Immunology Assays

1. Agglutination Reactions
   - to detect particulate antigens in solution
   - antibodies cause clumping (agglutination) of their specific antigens
   (e.g. Hemagglutination for blood typing: detects surface antigens on RBCs)
A. Direct agglutination tests
-to detect if patient has antibodies to particular antigen
  (antibodies present = exposure or infection by the agent)
-known infectious agents are bound to a microtiter dish
-patient serum is added
-if patient has antibodies to agent agglutination will occur
B. Indirect agglutination tests
- indirect = uses latex particles
- two ways:
  1. - known antigen is bound to latex particles
     - assay for patient sample for antibody
  2. - known antibodies are bond to latex particles
     - assay for antigenic agent

\(\text{(a)}\) Reaction in a positive indirect test for antibodies. When particles are coated with antigens, agglutination indicates the presence of antibodies, such as the IgM shown here.

\(\text{(b)}\) Reaction in a positive indirect test for antigens. When particles are coated with monoclonal antibodies, agglutination indicates the presence of antigens.
2. Fluorescent Antibody Labeling
-use antibody chemically linked to florescent dye that is visible with UV light

A. Direct FA Test
-identify or visualize microbes in clinical specimens
-probe specimen with fluorescent antibodies

A. Indirect FA Test
-detect the presence of antibodies in serum
-fix known antigen to slide, probe with patient serum, tag with fluorescent antihuman immune serum globin (αHISG)
antihuman immune serum globin (αHISG) -antibody that will bind to human antibodies at Fc region
-αHISG antigen binding sites are specific for any human IgG (or IgM) molecules as their epitope

-Fc region of αHISG will have fluorescent molecules or enzymes attached to allow detection/visualization of binding
3. ELISA
(Enzyme Linked ImmunoSorbant Assay)
-used to detect either antibodies or antigens in patient sample
-performed in microtiter plate
-positive reaction produces color change
-process can be automated: computer readout of many samples at once
-risk of false positives
A. Direct ELISA (“Sandwich ELISA”)

- antibody bound to dish
- assay for presence of antigen in sample

1. Antibody is adsorbed to well.

2. Patient sample is added; complementary antigen binds to antibody.

3. Enzyme-linked antibody specific for test antigen is added and binds to antigen, forming sandwich.

4. Enzyme's substrate (●) is added, and reaction produces a product that causes a visible color change (●).

(a) A positive direct ELISA to detect antigens
B. Indirect ELISA
- antigen bound to dish
- assay for presence of antibody in sample

1. Antigen is adsorbed to well.

2. Patient serum is added; complementary antibody binds to antigen.

3. Enzyme-linked anti-HISG (see page 513) is added and binds to bound antibody.

4. Enzyme's substrate (●) is added, and reaction produces a product that causes a visible color change (●).

(b) A positive indirect ELISA to detect antibodies
4. Western Blot / Immunoblotting
-often used to confirm ELISA positive result
-blot assays both size of protein antigen and specific reaction with antibody
-size confirmation proves/disproves possible cross reaction (false positive) method:
1) proteins collected from patient sample and separated by size on gel electrophoresis

-electric field drives proteins through gel: large stay near top, small move toward bottom
2) separated proteins are transferred (blotted) to a nylon membrane

3) membrane exposed to antiserum for suspect pathogen

4) antibodies in antiserum specifically bind to their epitope

5) a colored substrate is added: reacts with the Fc region of bound antibodies thus coloring location of antigen with bound antibody

6) pathogen is confirmed by:
   - binding of specific antiserum
   - size of the epitope
Lyme disease reactive Western blot

Description of lanes:
Lane 1 - molecular weight marker
Lane 2 - positive patient sample
Lane 3 - positive patient sample
Lane 4 - monoclonal antibodies for 39 and 41kD bands
Lane 5 - monoclonal antibodies for 41kD band
Lane 6 - monoclonal antibodies for 39 and 41kD bands
Lane 7 - monoclonal antibodies for 31 and 34kD bands
Lane 8 - positive control pool