- IgE is homocytotropic; that is, it has an affinity for cells ("cytotropic") of the host species that produced it ("homo"). This affinity is particularly strong for tissue mast cells and blood basophils. Fixation to these cells occurs via a **cell membrane bound $F_c\varepsilon R$** (i.e., receptor for the Fc portion of the **ε** chain of IgE) reacting with the Fc fragment (CH3and CH4domains).
- On combining with allergens, IgE antibodies trigger the release of histamine and other mediators of atopic disease from the cells.
- IgE may also be important in immunity to certain helminthic parasites.
- IgE is unable to activate complement via the classical pathway.
- IgE has a vascular half-life of 2 to 3 days and is heat-labile at 56°C

Production: IgE is produced by B cells and plasma cells in the spleen, in lymphoid tissue of the tonsils and adenoids, and in the respiratory and gastrointestinal mucosa. IgE does not cross the placenta. IgE production begins in the fetus early in gestation.

5. Immunoglobulin D (IgD):-

It was first discovered in a patient developed multiple myeloma. They have serum concentrate of 30 µg/ml (approximately 3 to 5 mg/dl).and constitute about .2% of human serum. They are present extensively on PM of B-cells. Their exact function is unknown but probably they play a role in Antigen dependent B-cells differentiation i.e. it eliminates B-cells which can produce self reactive antibodies**. IgD exists as a monomer**

CHAPTER: 17 MONOCLONAL ANTIBODIES

An antibody produced by a single clone of cells (specifically, a single clone of hybridoma cells) and therefore a single pure homogeneous type of antibody. Monoclonal antibodies can be made in large amounts in the laboratory and are a cornerstone of immunology. The term "monoclonal" pertains to a single clone of cells, a single cell and the progeny of that cell.

Köhler and Milstein found a way to combine

- the unlimited growth potential of myeloma cells with
- the predetermined antibody specificity of normal immune spleen cells.

They did this by literally fusing myeloma cells with antibody-secreting cells from an immunized mouse. The technique is called **somatic cell hybridization**. The result is a **hybridoma**

The procedure

Mix the following spleen cells from a mouse that has been immunized with the desired antigen with myeloma cells. Use an agent to facilitate fusion of adjacent plasma membranes like PEG. Even so, the success rate is so low that there must be a way to **select for** the rare successful **fusions.**

So, use myeloma cells that have:

- **lost** the ability to synthesize **hypoxanthine-guanine-phosphoribosyltransferase** (**HGPRT**).

This enzyme enables cells to synthesize purines using an extracellular source of **hypoxanthine** as a precursor. Ordinarily, the absence of HGPRT is not a problem for the cell because cells have an alternate pathway that they can use to synthesize purines.

However, ***when cells are exposed to aminopterin (a folic acid analog), they are unable to use this other pathway and are now fully dependent on HGPRT for survival.***

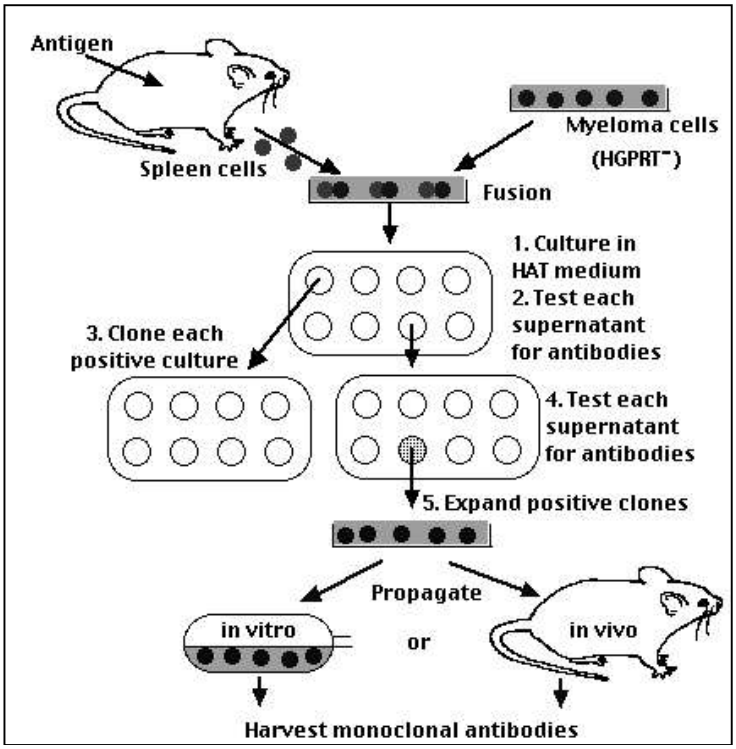
- **lost** the ability to synthesize any antibody molecules of their own (so as not to produce a hybridoma producing two kinds of antibody molecules).

1. The first property is exploited by transferring the cell fusion mixture to a culture medium - called **HAT medium** because it contains:

- **h**ypoxanthine
- **a**minopterin
- the pyrimidine **t**hymidine

The logic:

- Unfused myeloma cells cannot grow because they lack HGPRT.
- Unfused normal spleen cells cannot grow indefinitely because of their limited life span. However,
- Hybridoma cells (produced by successful fusions) are able to grow indefinitely because the spleen cell partner supplies HGPRT and the myeloma partner is immortal.



2. Test the supernatants from each culture to find those producing the desired antibody.

3. Because the original cultures may have been started with more than one hybridoma cell, you must now isolate single cells from each antibody-positive culture and subculture them.
4. Again, test each supernatant for the desired antibodies. Each positive subculture having been started from a single cell - represents a clone and its antibodies are monoclonal. That is, each culture secretes a single kind of antibody molecule directed against a single determinant on a preselected antigen.
5. Scale up the size of the cultures of the successful clones.
Hybridoma cultures can be maintained indefinitely:

- ***in vitro***; that is, in culture vessels. The yield runs from 10-60 µg/ml.
- ***in vivo***; i.e., growing in mice. Here the antibody concentration in the serum and other body fluids can reach 1-10 mg/ml. [When the hybridoma cells are injected in mice (in the peritoneal cavity, the gut), they produce tumors containing an antibody-rich fluid called **ascites fluid**.]

***Catalytic Antibodies*:** Antibodies have been selected for their affinity for the antigen rather than for the transition state of any reaction the antigen might undergo. If the immunogen were a transition state or a transition-state analogue, however, antibodies should catalyze the appropriate reaction. If this were the case, it should be possible to make antibodies with catalytic activity to order.
Monoclonal antibodies directed against various transition-state analogues have been found to have some of the expected catalytic activities and specificities. Such antibodies have many of the characteristics of enzymes in that they accelerate reactions up to 10^5-fold over the noncatalyzed rate, show comparable substrate specificities, exhibit a Michaelis K_m values for substrates, and are subject to competitive inhibition. The catalytic activities of the antibodies generated thus far are still lower than of natural enzymes, however, probably as a result of deficiencies in the way the analogues mimic the true transition states.
Other approaches can be taken to generate catalytic antibodies. Bisubstrate inhibitors can generate antibodies that bind the two individual substrates and enhance reaction between them simply due to their proximity in the antibody combining sites. Reactive groups can be generated in combining sites by using the appropriate immunogen, by mutation of the antibody gene, or by chemical modification of the antibodies.

CHAPTER: 18 ANTIGEN–ANTIBODY INTERACTIONS

I. *IN VITRO* ANTIGEN-ANTIBODY REACTIONS (i.e., serologic reactions) provide methods for the diagnosis of disease and for the identification and quantitation of antigens and antibodies.

A. The titer, or level, of antibody in serum can be measured by using known antigens, and such titers can be of diagnostic and prognostic importance (e.g., a rise in antibody titer between acute and convalescent serum can be diagnostic for a specific disease). The titer of an antiserum usually is obtained by determining the greatest dilution of serum that reacts with the antigen.

B. The forces involved in antigen-antibody interactions are profoundly affected by various environmental factors. The antigen-antibody complex is not bound firmly together; it may even dissociate spontaneously. However, the equilibrium is far to the right, with a very large association constant (Kd) of 10^{-6} to 10^{-8}.
1. Physiologic pH and salt concentration promote optimal union. Forces of attraction tend to be weaker in very acidic (i.e., below pH 4.0) and alkaline (i.e., above pH 10.0) conditions. High salt concentrations also can inhibit the interaction between an antigen and its homologous antibody.
2. Temperature also plays an important role: The higher the temperature (up to a maximum of 50°-55°C), the more rapid is the rate of reaction. This is due to the increase in kinetic motion of the reactants.

C. Various forces act to hold the antigen-antibody complex together. The maximum attractive forces stabilizing antigen-antibody complexes are Van der Waals forces and ionic bonds. .
Van der Waals forces occur because of spatial fit. These forces hold antigen to antibody when the two molecules have complementary shapes. When the molecules have less similar shapes, these forces are less effective
2. Ionic bonds (also called coulombic forces) are patterns of complementary electrical charge on the molecule. The electrostatic interactions tend to hold the molecules together.

D. Affinity. The strength of the attraction between a single epitope and its matching paratope (the antigen-binding site on the antibody molecule) is referred to as the affinity of the reaction between the two reactants. Antigen-antibody complexes of low affinity dissociate readily.

E. Avidity, refers to the strength of the interaction between multivalent antigens and the population of antibodies that they have induced. **Avidity is influenced by the affinity of individual antibodies for their epitopes, the valence of the antigen, and the valence of the antibodies**.

F. Studies using synthetic polypeptides have shown that only those amino acids that are spatially accessible because of tertiary protein structure are immunoreactive.
1. Proteins can exist as globular or fibrous proteins or mixtures of both; the nature of the structure is important.
2. The ability of antibody to bind to antigenic sites can be affected by altering the tertiary structure.
a. The antigenic sites then would no longer be spatially arranged in such a way that antibody-antigen coupling could occur.

b. Insulin molecules provide an illustration.

►Insulin is composed of A and B chains. Antibody to either one of these chains can be produced by splitting the chains, purifying them, and injecting them into a foreign host (e.g., a pig). The host produces antibody to the particular chain that was injected.

►If the host's (i.e., the pig's) antibodies are injected back into the animal species that s.upplied the original insulin (i.e., man), the antibodies will not react with intact insulin molecules.

►The tertiary structure of native insulin is such that the epitopes on the A and B chains are not accessible.

G. The physical state of the antigen is responsible, in general, for the identification of antigen-antibody reactions and the naming of antibodies. The same antibody molecule could, in fact, be described by each of the following terms.
1. Agglutinins are antibodies that aggregate cellular antigens.
2. Lysins are antibodies that cause dissolution of cell membranes.
3. Precipitins are antibodies that form precipitates with soluble antigens.
4. Antitoxins are antibodies that neutralize toxins.

PRECIPITATION REACTIONS

►The interaction between an antibody and a soluble antigen in aqueous solution forms a lattice that eventually develops into a visible precipitate. Antibodies that thus aggregate soluble antigens are called precipitins.

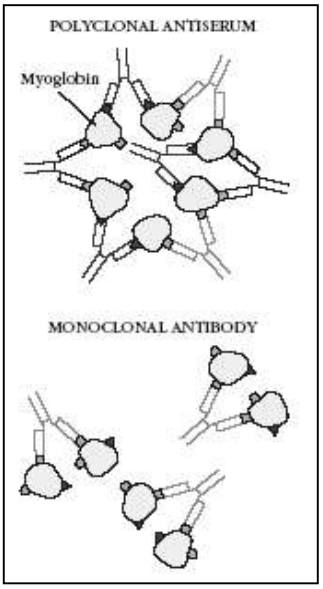
►The precipitate develops as neighboring antibody molecules within the lattice form ionic bonds with each other, causing the lattice to lose its charge and thus become insoluble.

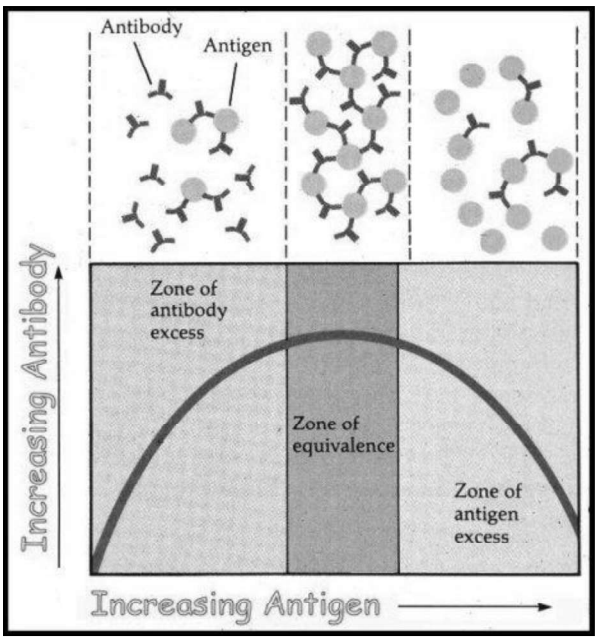
Formation of an Ag-Ab lattice depends on the valency of both the antibody and antigen:

- The antibody must be bivalent; a precipitate will not form with monovalent Fab fragments.
- The antigen must either be 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.

Precipitation reactions.

- (a) **Polyclonal antibodies can form lattices, or large aggregates, that precipitate out of solution**. However, if each antigen molecule contains only a single epitope recognized by a given monoclonal antibody, the antibody can link only two molecules of antigen and no precipitate is formed.
- (b) **A precipitation curve for a system of one antigen and its antibodies.** This plot of the amount of antibody precipitated versus increasing antigen concentrations (at constant total antibody) reveals three zones: a zone of antibody excess, in which precipitation is inhibited and antibody not bound to antigen can be detected in the supernatant; an equivalence zone of maximal precipitation in which antibody and antigen form large insoluble complexes and neither antibody nor antigen can be detected in the supernatant; and a zone of antigen excess in which precipitation is inhibited and antigen not bound to antibody can be detected in the supernatant.





PRECIPITATION REACTIONS IN FLUIDS

►A quantitative precipitation reaction can be performed by placing a constant amount of antibody in a series of tubes and adding increasing amounts of antigen to the tubes. After the precipitate forms, each tube is centrifuged to pellet the precipitate, the supernatant is poured off, and the amount of precipitate is measured.

►Plotting the amount of precipitate against increasing antigen concentrations yields a precipitin curve. Excess of either antibody or antigen interferes with maximal precipitation, which occurs in the so-called equivalence zone, when the ratio of antibody to antigen is optimal. As a large multimolecular lattice is formed at equivalence, the complex increases in size and precipitates out of solution.

►In the region of antibody excess, unreacted antibody is found in the supernatant along with small soluble complexes consisting of multiple molecules of antibody bound to a single molecule of antigen.

►In the region of antigen excess, unreacted antigen can be detected and small complex are again observed, this time consisting of one or two molecules of antigen bound to a single molecule of antibody.

PRECIPITATION REACTIONS IN GELS

RADIAL IMMUNODIFFUSION (MANCINI METHOD)

►The relative concentrations of an antigen can be determined by a simple quantitative assay in which an antigen sample is placed in a well and allowed to diffuse into agar containing a suitable dilution of an antiserum. As the antigen diffuses into the agar, the region of equivalence is established and a ring of precipitation forms around the well. The area of the precipitin ring is proportional to the concentration of antigen.