BLOOD BANK

Immunity

- process by which a host **organism protects itself** from attacks by external and internal agents

Antigen and Antibody

Antigen

- A substance that on introduction gives rise to a formation of antibodies that reacts specifically with the same antigenic substance

Antibody

- A **gamma-globulin** product of an immune response, (also called immunoglobulin) that reacts with antigen against which it is stimulated

Characteristics of Antigen

Chemical Structure of Antigen

- Antigens composed of oligosaccharides tend to stimulate IgM type of antibody production
- Antigens which are primarily protein in nature, produce IgG antibody

Degree of Foreignness

Number of Antigens Introduced

- Higher the dose, greater the antibody production

Route of administration

- Intramuscular or subcutaneous route more potent

Immunogenicity

- Ability of an antigen to stimulate the production of its corresponding antibody in a person who lacks the antigen
- Rh D most potent antigenic

- Fy^a is poor antigenic
- *Various characteristics of an antigen decides the immunogenicity of that particular antigen.*
- *The chemical structure of the antigen determines the type of antibody response.*
- *Similarly, more the antigen foreign in nature, that is non-self, more will be the immunogenicity of that antigen.*
- *Immunogenicity - is the ability of foreign substance, such as antigen to provoke an immune response in the body of a human or other animal*

Table 3–7 Relative Immunogenicity of Different Blood Group Antigens

BLOOD GROUP ANTIGEN	BLOOD GROUP SYSTEM	IMMUNOGENICITY (%)*
D (Rh_o)	Rh	50
K	Kell	5
c (hr′)	Rh	2.05
E (rh″)	Rh	1.69
k	Kell	1.50
e (hr″)	Rh	0.56
Fy^a	Duffy	0.23
C (rh′)	Rh	0.11
Jk^a	Kidd	0.07
S	MNSs	0.04
Jk^b	Kidd	0.03
s	MNSs	0.03

Immunoglobulins

- Five types, viz. **IgG, IgM, IgA, IgD & IgE.**
- Blood group antibodies are mainly confined to IgG & IgM.
- IgG is a smaller molecule, with a pair each of heavy & light chains of amino acids.

- IgG antibodies can just coat but not agglutinate the cells.
- IgM has 5 such pairs joined together by the J chain.
- IgM antibodies agglutinate the cells bearing corresponding antigen.
- *IgA - secretory form*
- *IgE- allergy*
- *IgD - activation of the immune system,expressed in the plasma membrane of immature B lymphocytes.*

Properties	IgG	IgM	IgA
H chain, Class	Gamma	Mu	Alpha
Serum conc (mg/dL)	1000 – 1500	85 - 205	200 - 350
Molecular weight	150,000	900,000	180,000
Complement fixation	Occasionally	Yes	No
Structure	Monomer	Pentamer	Mono / diamer
Crosses placenta	Yes	No	No
Secretions	No	No	Yes

IgM Antibodies (Complete)

- Agglutinate in saline phase
- Pentameric - Decavalent
- Usually naturally occurring
- Do not cross placenta
- React at temperature varying from 4 – 20°C
- **Example: ABO antibodies**
- *IgM antibodies agglutinate red cells in saline phase at room temperature.*
- *They are pentavalent in structure.*
- *They are naturally occurring, meaning that they do not need any antigenic stimulus and are present from birth.*
- *They do not cross placenta.*
- *Classical example of such type of antibodies is antibodies against ABO antigens*

IgG Antibodies (Incomplete)

- Agglutinate in IAT phase
- However, may cause agglutination in saline phase using albumin / enzymes
- Monomeric - Bivalent
- Usually immune in nature
- Can cross placenta
- React at 37°C
- **Example: Rh antibodies**
- *IgG antibodies are called incomplete antibodies as they do not cause agglutination of red cells in the saline phase and require AHG to cause agglutination.*
- *They are monovalent in structure and require some form of immunologic stimulus for their production.*
- *Because of their small size, they can easily cross placenta.*
- *Classical example of such antibodies is Rh antibodies seen in immunized Rh negative mother*
- *IAT - indirect antiglobulin test*

IgG antibodies can bridge a gap of about 14 nm, while IgM antibodies, because of their pentameric structure, can bridge the gap of 35 nm. Red cells are separated from each other because of surface negative charge. The distance between two red cells is about 25 nm. This explains why IgG antibodies can not cause agglutination of red cells without adding AHG

***Primary Response**: Results upon introduction of the antigen. IgM antibodies develop slowly and in small amounts.*

***Secondary Response**: Results to subsequent exposures of the same antigen. IgG antibodies are produced in large amounts in a short period, usually within 48 hours.*

Naturally occurring Vs Immune Antibodies

Feature	Naturally occurring	Immune
Antigen stimulus	Obscure, possibly from microbial origin	Human red cell antigens
Type of Immunoglobulin	IgM	IgG
Optimum temperature	< 22° C	at 37° C
Clinical significance	Acute HTR	HDN, DHTR
Examples	ABO antibodies	Rh, Kell, Kidd, Duffy antibodies

Types of Antigen-Antibody Reactions

- Agglutination
- Sensitization
- Hemolysis
- Neutralization (inhibition)
- Precipitation
- Immunofluorescence
- Complement fixation
- Radio Immunoassay

Red cells are possessing antigens on their surface. Serum of an individual contains antibody. If the red cells are added to this serum, there will be antigen antibody reaction which may or may not cause complement activation. If complement is activated, it will result in hemolysis.

Stages of ag-ab reaction

Stage of Sensitization

- Only coating of red cells with IgG antibody without causing agglutination
- Bond holding Ag-Ab complex may dissociate & re-associate till the state of equilibrium is reached

Stage of Agglutination

- Characterized by formation of bridges between sensitized red cells resulting in visible aggregate of red cells

There are two stages of antigen antibody reaction. In the stage of sensitization, IgG antibodies attach to the red cell membrane. At this stage antigen antibody complex may dissociate from the red cells. In the stage of agglutination, there is bridging of the gaps between red cells resulting in visible agglutination

Factors Affecting Stage of Sensitization

Antigen – Antibody ratio

- Two volume of serum and one volume of 5% red cells
- Sensitivity of test depends upon number of antibody molecules bound per red cells

pH

- Most antibodies are detected at neutral pH

Temperature

- IgM antibodies react optimally at cold temp while IgG at 37°C

Incubation Time

- Time needed to reach Ag-Ab reaction at equilibrium
- Too short incubation – weaker reaction

- Prolonged incubation results in dissociation of antibody

Factors Affecting Stage of Agglutination

Size & Class of Antibody

- IgM antibody, being a pentamer can bind antigen sites up to 35 nm apart
- IgG antibody, being a monomer can bind antigen sites up to 14 nm apart

Antigenic Sites

- Antigens located on surface of red cell membrane (ABO) result in strong agglutination
- Antigens embedded in membrane (Rh antigens) result in weaker agglutination

Zeta Potential

- Electrostatic repulsive force between red cells

Red cells have a negative surface charge and therefore repel each other and attract positive charge sodium ions when suspended in normal saline. This results in the formation of a double layer ionic cloud around red cells. The difference in charge density between the inside and the outside of the ionic cloud creates an electrostatic potential known as Zeta potential.

Effect of Adding LISS

- Reduction in zeta potential using LISS
- RBCs come closer together
- Strong agglutination
- Reduction in incubation time
- *Zeta potential may be reduced by potentiating the medium with albumin and enzyme, thereby decreasing the repulsion between cells and allowing them to approach one another more closely and permit agglutination. Thus, upon addition of LISS, Zeta potential is reduced allowing RBCs to come closer and bring about strong agglutination.*

Red Cell Serological Techniques

- ABO and Rh (D) typing
- Weak ‘D’ or D^u testing
- Antibody screening & identification
- Pre-transfusion testing
- Indirect antiglobulin test
- Direct antiglobulin test