Blood Groups:

Genetic transmission of antigens; Erythrocyte antigens; Blood group nomenclature, Anti-erythrocyte antibodies

- A **blood group** is a “classification” of blood based on the presence or absence of inherited antigenic substances on the surface of red blood cells (RBCs).
- These antigens may be proteins, carbohydrates, glycoproteins, or glycolipids, depending on the blood group system.
- Some of these antigens are also present on the surface of other types of cells of various tissues or in some secretions.
- ***Also called blood type***

- Many Blood group systems exist

e.g: ABO, Rhesus, Kell, Lewis, etc

- Blood group systems are Ags based
- These Ags are transmitted from parents to offspring
- Principles of genetics apply

e.g: ABO BLOOD GROUP SYSTEM

- History
  1. Landsteiner discovered the ABO Blood Group System in 1901 (1900)
  2. He and five co-workers began mixing each others red blood cells and serum together and accidentally performed the first forward and reverse ABO groupings.
  3. Landsteiner's Rule: If an antigen (Ag) is present on a patient's red blood cells the corresponding antibody (Ab) will NOT be present in the patient's plasma, under 'normal conditions'.

ABO INHERITANCE

T2

Dad = *A/O* and Mom = *B/O*		Mom	
		B	*O*
Dad	*A*	A/B	A/O
	O	O/B	O/O

ABO groups of the offspring from the various possible ABO mating

Phenotypes (Parents)	Genotype	offspring
AxA	AAxAA	A (AA)
	AAxAO	A (AA or AO)
	AOxAO	A (AA or AO),O(OO)
BxB	BBxBO	B (BB or BO)
AxAB	AAxAB	AB (AB) or A (AA)
	AOxAB	AB (AB), A (AA, AO), B(BO)

SUB GROUPS EXIST

e.g: A1 and A2

- The A blood type contains about twenty subgroups, of which A1 and A2 are the most common (over 99%).
- A1 makes up about 80% of all A-type blood, with A2 making up the rest.
- These two subgroups are interchangeable as far as transfusion is concerned, but complications can sometimes arise in rare cases when typing the blood

- Definition

  Isoagglutinins: are defined as antibodies that agglutinate blood cells of some individuals of the same species

  Glycosyltransferases: are enzymes that facilitate the transfer of carbohydrate (sugar) molecules onto carbohydrate precursor molecules

  Immunodominant sugar: is the sugar molecule that complete the antigenic determinant when combined with the precursor substance

ABH ANTIGEN

- The inheritance of the ABO blood group was demonstrated that each individual inherits one ABO gene from each parent and these two genes determine which Ags are present on RBCs membrane

- One position or Locus, on each chromosome ***number nine*** is occupied by an A, B, or an O gene

ABH Antigens

- Ags belonging to ABH blood group system are present on RBCs and other body cells and body fluids.

- The presence of A,B, and O Ags on RBCs depends upon the allelic genes, A,B, and O
- An H gene at a separate locus codes for the precursor substance on which the A and B gene products act.
- The products of the A and B genes are enzymes that act as specific transferases

ABH Antigens

- H gene products is an enzyme that produce H substance
- The O gene is a silent allele
- It does not alter the structure of H substance.

Formation of A,B& H Antigen

- The ABO genes do not code for the production of ABO antigens, BUT rather produce specific transferases
- ABO produce specific transferases that add sugars to a basic precursor substance on the RBCs

Formation of A,B& H Antigen

- The inheritance of at least one H gene (HH or Hh) elicits (obtain) the production of an enzyme called, α-2-L-Fucosyl transferase, which transfers the sugar from the Guanosine diphosphate L-fucose (GDP-Fuc) donor nucleotide to the terminal galactose of the precursor chain.

- The H substance must be formed for the other sugars to be attached in response to an inherited A and /or B genes

ABH Ag

There are two potential precursors substance (PS) both are comprised of identical sugar (galactose-N- acetyl glucossamin - galactose -glucose) but different in linkage.

- Type I PS has a terminal galactose (Gal) linked to a sub-terminal N-acetyl-glucosamine (GlcNAc) in 1-3 linkage

- Type II PS, has the same sugar combine in 1-4 linkage

ABO Genetics

- Genes at three separate loci control the occurrence and location of A and B antigens

1. *Hh* genes – *H* and *h* alleles
   - *H* allele codes for a fucosyltransferase enzyme that adds a fucose on Type 2 chains (primarily) to form the H antigen onto which A and B antigens are built on red blood cells.
   - *h* allele is a silent allele

ABO Genetics

- A, B and H antigens are built on oligosaccharide chains of 4 types. The most common forms are Type 1 and Type 2.

Type 1: #1 carbon of Gal is attached to the #3 carbon of GlcNAc.

Type 2: #1 carbon of Gal is attached to the #4 carbon of GlcNAc.

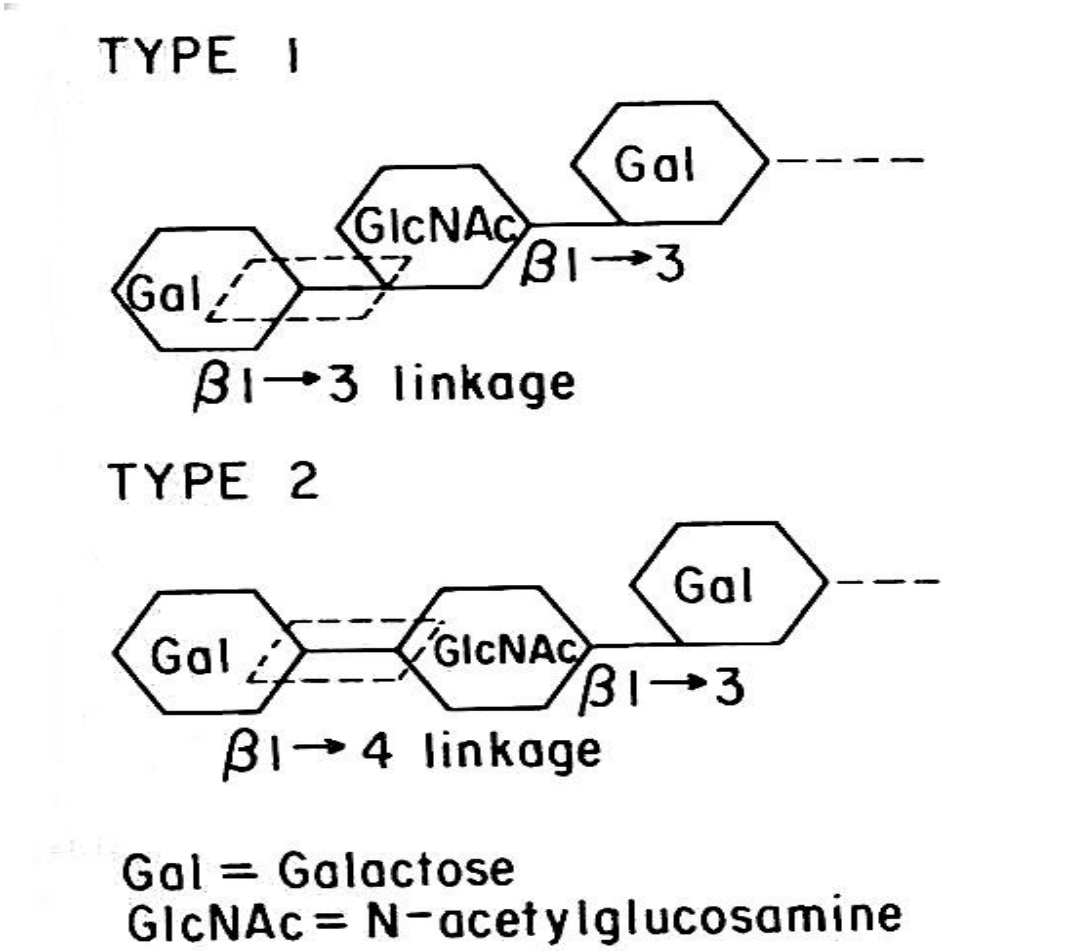


Figure 13-2. Type 1 and 2 oligosaccharide chains differ only in the linkage between the GlcNAc and the terminal Gal.

ABO Genetics

2- *Se* genes – *Se* and *se* alleles

- *Se* allele codes for a fucosyltransferase enzyme that adds fucose onto Type 1 chains (primarily) in secretory glands. Controls expression of H antigens in secretions (i.e. saliva, body fluids, etc.)
- *se* allele is an a morph

3. *ABO* genes – *A*, *B* and *O* alleles

- *A* and *B* alleles code for (glycosyltransferases) fucosyltransferase enzymes that add a sugar onto H antigens to produce A and B antigens
- *O* allele does not code for a functional enzyme

ABO Genetics

1. **Occurrence**
   a. The presence or absence of the ABH antigens on the red blood cell membrane is controlled by the *H* gene
   b. Presence or absence of the ABH antigens in secretions is indirectly controlled by the *Se* genes.

ABO Genetics

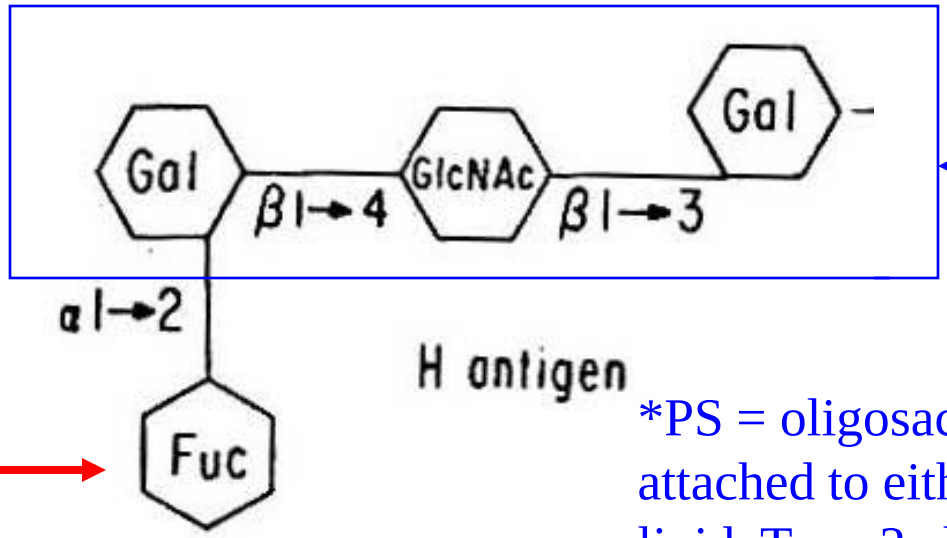
1. *Hh* gene – *H* and *h* alleles (*h* is an a morph)
2. *Se* gene – *Se* and *se* alleles (*se* is an amorph)
3. *ABO* genes– *A*, *B* and *O* alleles

1. Controls presence of H, A, and B antigens on both RBCs and in Secretions
2. Controls presence of H antigen in the secretions
3. Inherit 1 gene from each parent that codes for an enzyme that adds a sugar to the H antigen

H Antigen

The *H* gene codes for an enzyme (fucosylytranferase) that adds a Fucose to the terminal sugar of a Precursor Substance (PS*). The biochemical structure below constitutes the H Antigen. (*h* gene is an amorph.)

H gene acts on a Precursor substance(PS)* by adding **Fucose**



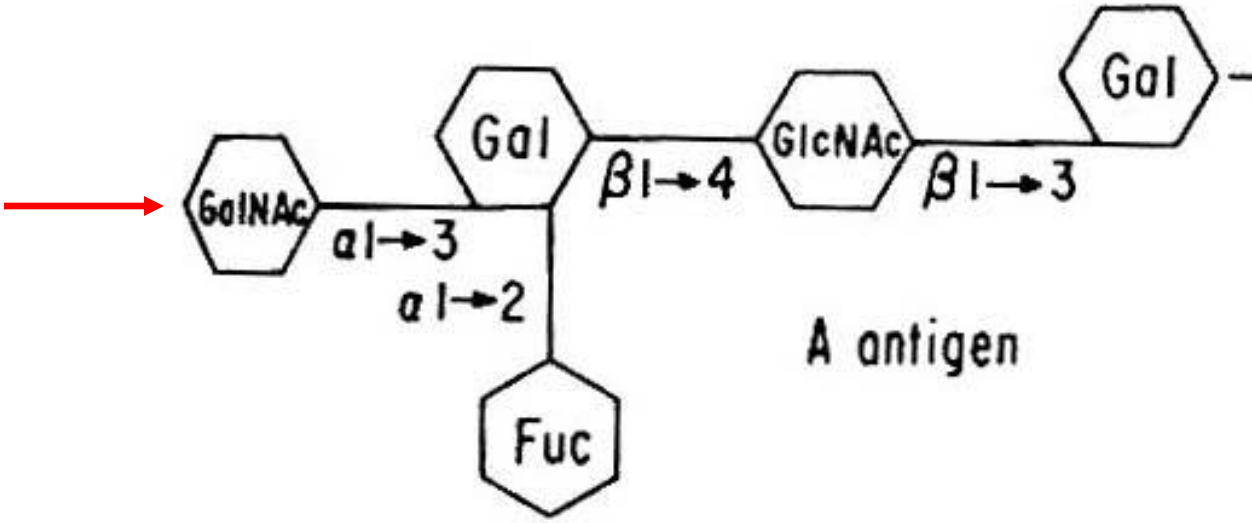
*PS = oligosaccharide chain attached to either glycosphingo-lipid, Type 2 chain (on RBC) or glycoprotein, Type 1 chain (in secretions)

ABO Genetics

- H antigen is the foundation upon which A and B antigens are built.
- *A* and *B* genes code for enzymes that add an immunodominant sugar to the H antigen.

Formation of the A Antigen

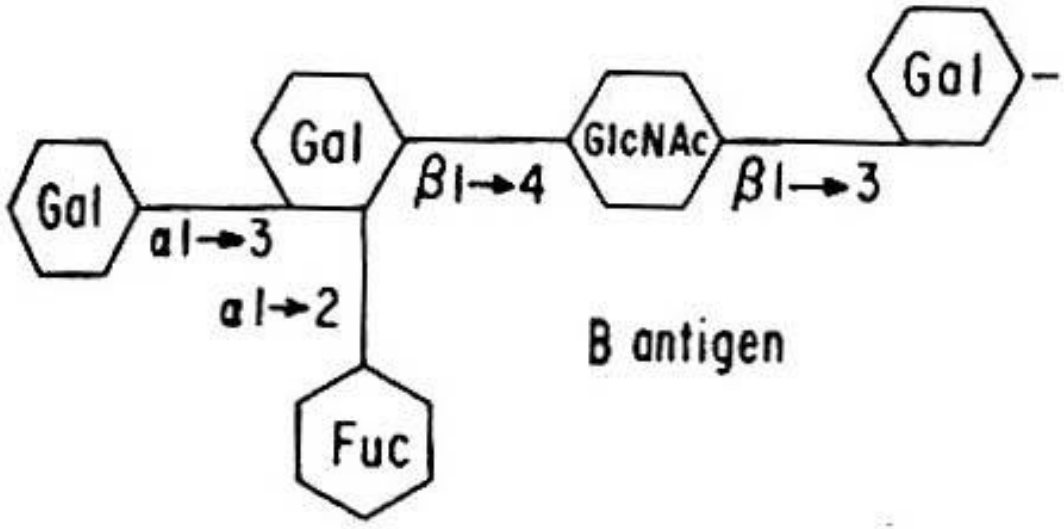
The **A gene** codes for an enzyme that adds GalNAc (N-Acetyl-D galactosamine) to the terminal sugar of the H Antigen.



The biochemical structure constitutes the A antigen.

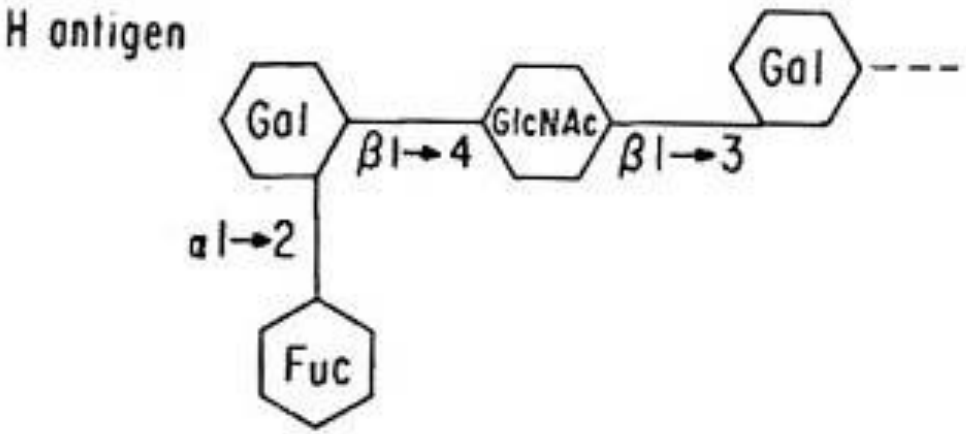
Formation of the B Antigen

B gene codes for an enzyme that adds D-Galactose to the terminal sugar of the H Antigen.

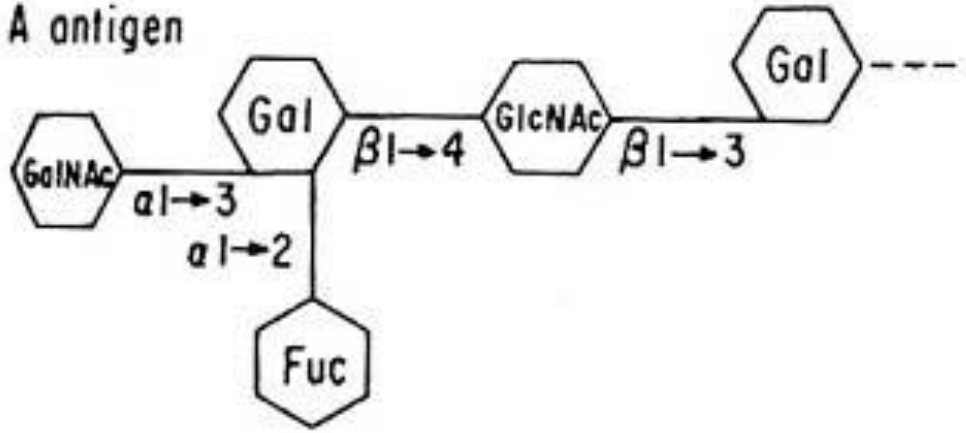


The biochemical structure constitutes the B Antigen.

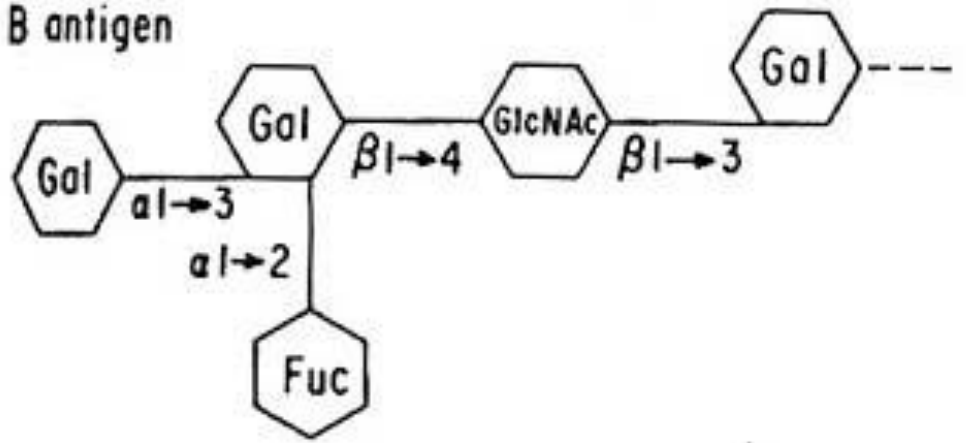
The **H antigen** is found on the RBCs when there is an *Hh* or *HH* genotypes but **NOT** with the *hh* genotype.



The **A antigen** is found on the RBCs when there is *Hh*, *HH*, and *A/A*, *A/O or A/B* genotypes.



The **B antigen** is found on the RBCs when there is *Hh*, *HH*, and *B/B*, *B/O or A/B* genotypes.



Amount of H Antigen According to Blood Group

- Blood Group O people have red blood cells rich in H antigen. Why?

Neither the A or B genes have converted the H antigens to A or B antigens - just a whole bunch of H!

O allele at the ABO locus (amorph)
It does not alter the structure of H substance.

Greatest Amount of H

$O > A_2 > B > A_2B > A_1 > A_1B$

Least Amount of H

Immunodominant Sugars and transferases responsible for H, A, and B Ags specificity

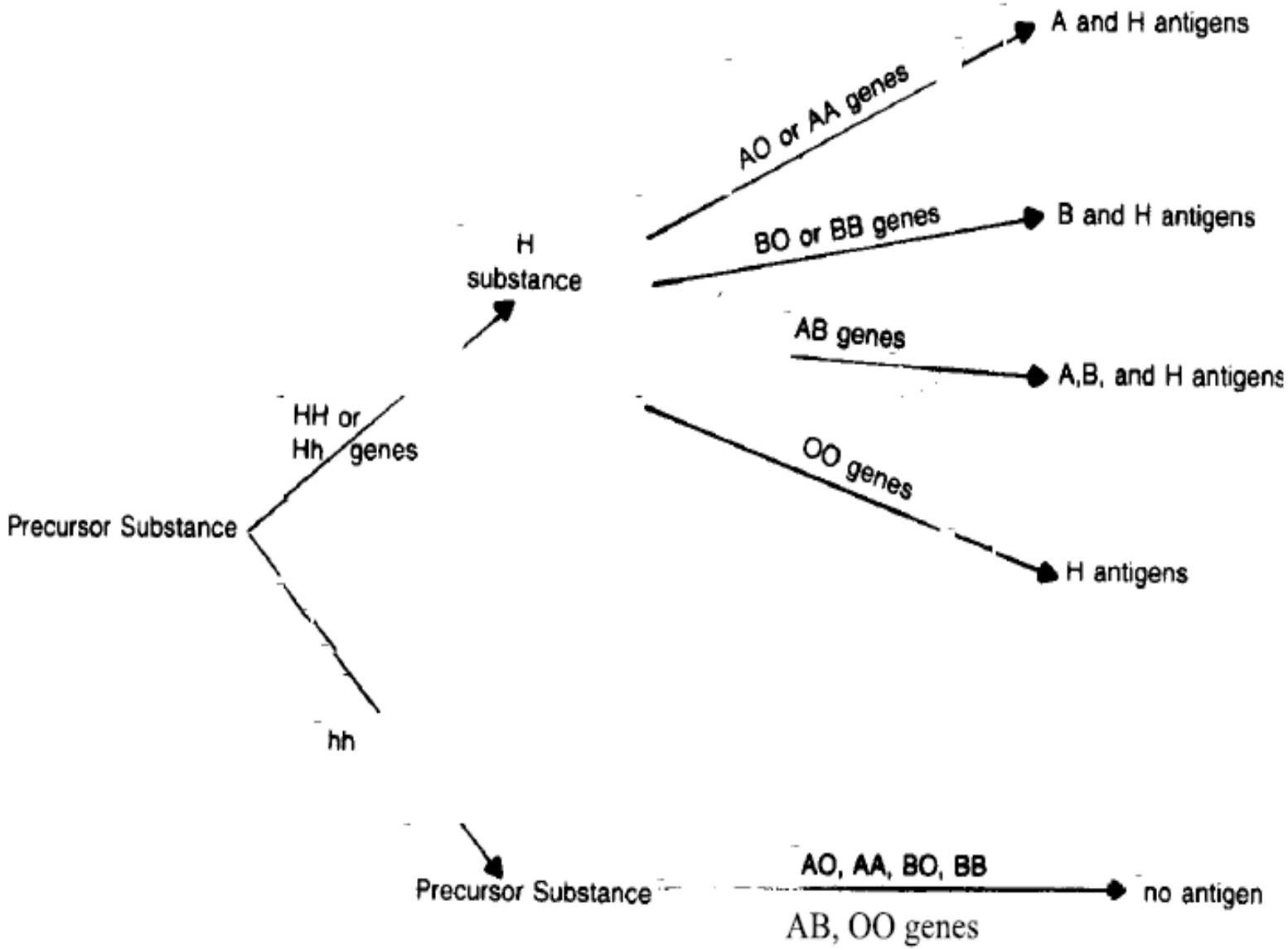
Gene	Glcosyltransferase	Immunodominant sugar	Antigen
H	**L- fucosyl trnsferas**	L-fucose	H
A	**N acetylgalactosaminyl transferase**	N-acetyl-D-galactoseamine	A
B	D- galactosyl transferase	D-galactose	B

Antibodies in ABO system

- The associated anti-A and anti-B antibodies are usually IgM antibodies (IgG are produced (some Ig A??), which are usually produced in the first years of life by sensitization to environmental substances such as food, bacteria, and viruses.
- ABO blood types are also present in some other animals, for example chimpanzees, and gorillas.

Bombay phenotype (hh genotype)

- Individuals with the rare Bombay phenotype (*hh*) do not express antigen H on their red blood cells.
- H antigen serves as precursor for producing A and B antigens
- The absence of H antigen means the individuals do not have A or B antigens as well (similar to O blood group).
- Unlike O group, Bombay Phenotype individuals produce isoantibodies to antigen H as well as Abs to both A and B antigens.
- In case they receive blood from O blood group, the anti-H antibodies will bind to H antigen on RBC of donor blood and destroy the RBCs by complement-mediated lysis.
- Therefore Bombay phenotype can receive blood only from other *hh* donors (although they can donate as though they were type O).



Genetic pathway of expression of ABH substances on erythrocytes.

- *TWO important principles about ABO BGS*

1. Almost all normal healthy individuals above 3-6 months of age have " naturally occurring Abs" to the ABO Ags that they lack

These Abs termed naturally occurring because they were thought to arise without stimulation by RBC antigens

2. These "naturally occurring" Abs are mostly of IgM class (capable of agglutinating saline/ low protein suspended red cell without enhancement and may activate complement cascade).

Major ABO Blood Group

Forward blood grouping using anti-sera and red blood cells

ABO Group	Antigen Present	Antigen Missing	Antibody Present
A	A	B	Anti-B
B	B	A	Anti-A
O	None	A and B	Anti-A&B
AB	A and B	None	None

If an antigen (Ag) is present on a patient's red blood cells , the corresponding antibody (Ab) will NOT be Normally present in the patient's plasma, under 'normal conditions'.

ABO BLOOD GROUP (Forward blood grouping)

Determination of ABO antigens found on patient red blood cells using reagent anti-sera. Serum from BG B aggl A RBCs, that an Ab to A Ag was present in Grp B serum, serum from A agg Grp B RBCs

Patient Red Cells Tested With			
Patient	**Anti-A**	**Anti-B**	**Interpretation**
1	0	0	O
2	+	0	A
3	0	+	B
4	+	+	AB

Reverse Grouping (Confirmatory grouping)

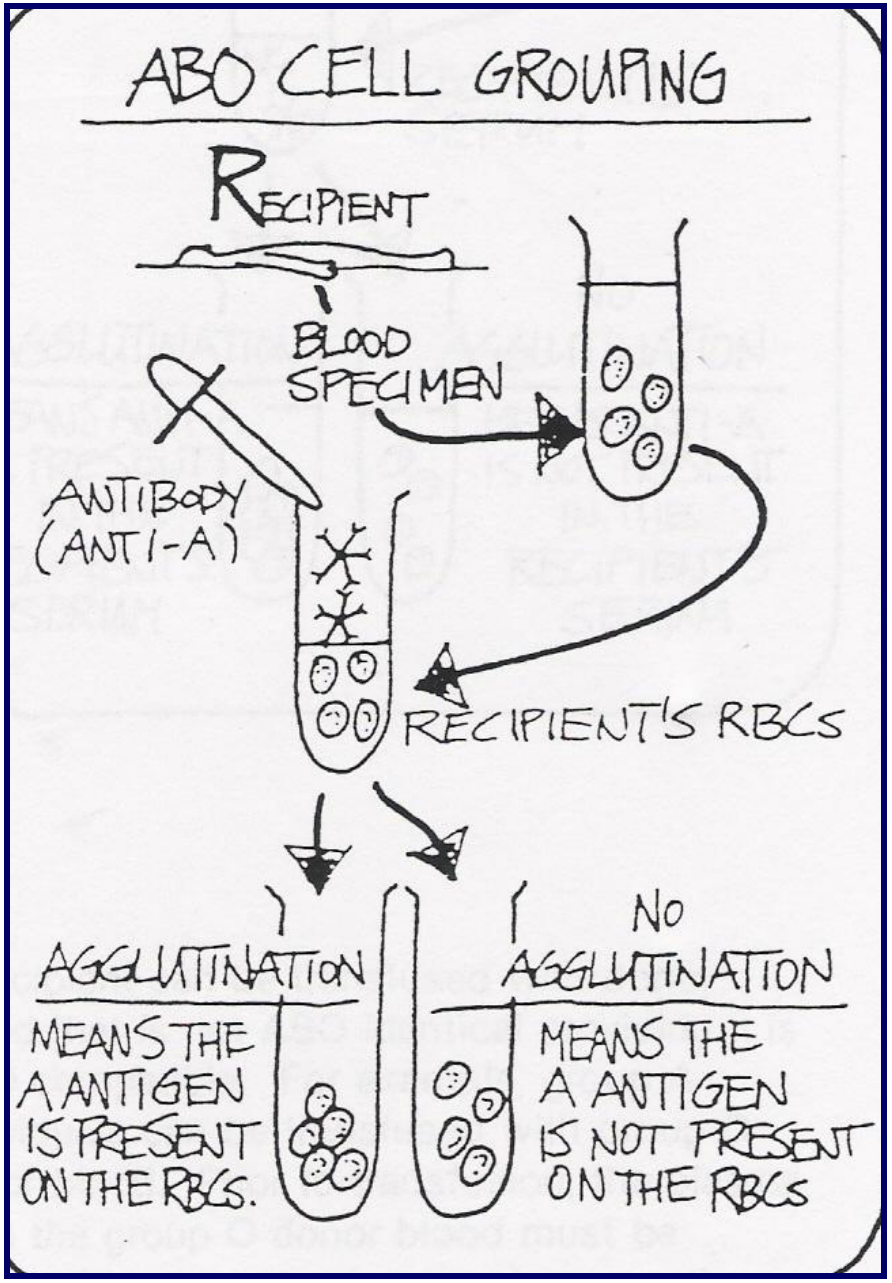
Patient Serum Tested With reagent red blood cells

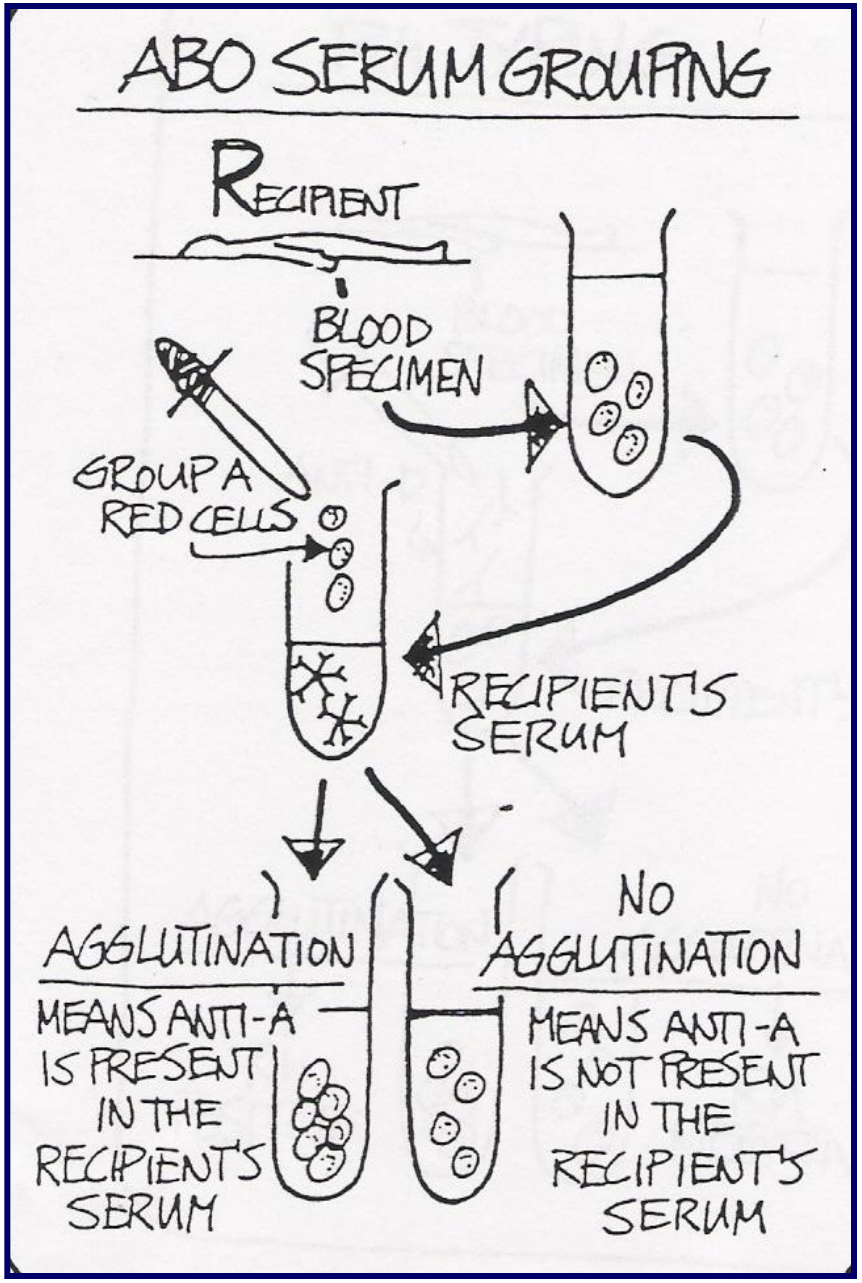
Serum from GRP O individual aggl both A and B cells indicate the presence of Abs to both A and B in group O serum

Patient	A Cells	B Cells	Interpretation
1	+	+	O
2	0	+	A
3	+	0	B
4	0	0	AB

FORWARD & REVERSE ABO BLOOD GROUPING

	Reaction of Cells Tested With		Reaction of Serum Tested Against		ABO Group
	Anti-A	Anti-B	A Cells	B Cells	
1	0	0	+	+	O
2	+	0	0	+	A
3	0	+	+	0	B
4	+	+	0	0	AB


ABO CELL GROUPING
RECIPIENT
BLOOD SPECIMEN
ANTIBODY (ANTI-A)
RECIPIENT'S RBCS
AGGLUTINATION
MEANS THE A ANTIGEN IS PRESENT ON THE RBCS.
NO AGGLUTINATION
MEANS THE A ANTIGEN IS NOT PRESENT ON THE RBCS


ABO SERUM GROUPING
RECIPIENT
BLOOD SPECIMEN
GROUP A RED CELLS
RECIPIENT'S SERUM
AGGLUTINATION
MEANS ANTI-A IS PRESENT IN THE RECIPIENT'S SERUM
NO AGGLUTINATION
MEANS ANTI-A IS NOT PRESENT IN THE RECIPIENT'S SERUM

Human Blood Groups

- RBC membranes have antigens on their external surfaces
- These antigens are:
  - Recognized as foreign if transfused into another individual lacking them.
  - Promoters of agglutination and are referred to as agglutinogens
- Presence or absence of these antigens is used to classify blood groups

Blood Groups

- More than 30 blood group systems exist
- The antigens of the ABO and Rh blood groups cause vigorous transfusion reactions when they are improperly transfused
- Other blood groups (Dufy, Kell, and Lewis, etc) are mainly used for legalities

ABO Blood Groups

- The ABO blood groups consists of:
  - Two antigens (A and B) on the surface of the RBCs
  - Two antibodies in the plasma (anti-A and anti-B)
  - ~~***""Antigen H"" from which A & B are produced***~~
- An individual of A, B, O blood grps may have various types of antigens and spontaneously preformed antibodies
- Agglutinogens and their corresponding antibodies cannot be mixed without serious hemolytic reactions

ABO Blood Groups

TABLE 17.4 ABO Blood Groups

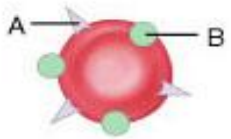
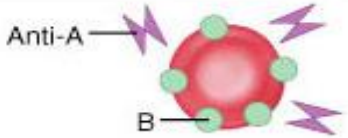
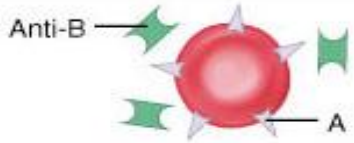
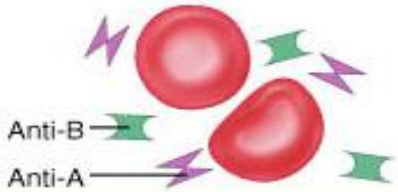
Blood Group	Frequency (% U.S. Population) White	Black	Asian	Native American	RBC Antigens (Agglutinogens)	Illustration	Plasma Antibodies (Agglutinins)	Blood That Can Be Received
AB	4	4	5	<1	A B		None	A, B, AB, O Universal recipient
B	11	20	27	4	B		Anti-A (a)	B, O
A	40	27	28	16	A		Anti-B (b)	A, O
O	45	49	40	79	None		Anti-A (a) Anti-B (b)	O Universal donor

Table 17.4

Rh Blood Groups

- Presence of the Rh agglutinogens on RBCs is indicated as Rh^+; 85% of population is +
- Lack of antigen indicated as Rh -; 15% of popn.
- If an Rh^- individual receives Rh^+ blood, anti-Rh antibodies form (allo-Abs)
- A second exposure to Rh^+ blood will result in a typical transfusion reaction

Blood Grp Nomenclature

E.g: EUROPE

- In parts of Europe, the "O" in ABO blood type is substituted with "0" (zero), signifying the lack of A or B antigen.
- In the former USSR blood types are referenced using numbers and Roman numerals instead of letters (I, II, III, and IV are elsewhere designated, respectively, as O, A, B, and AB).

Immune Response in BT

Introduction

- *Primary Immune response:* ***first contact with the Ag. No specific Abs already there.***
- ***Difficult to know the primary immune response to some Ags especially those of ABO system (natural Abs)***
- *Non respondent (never reacts) and poor respondent (reacts after many exposures) in Rhesus system.*

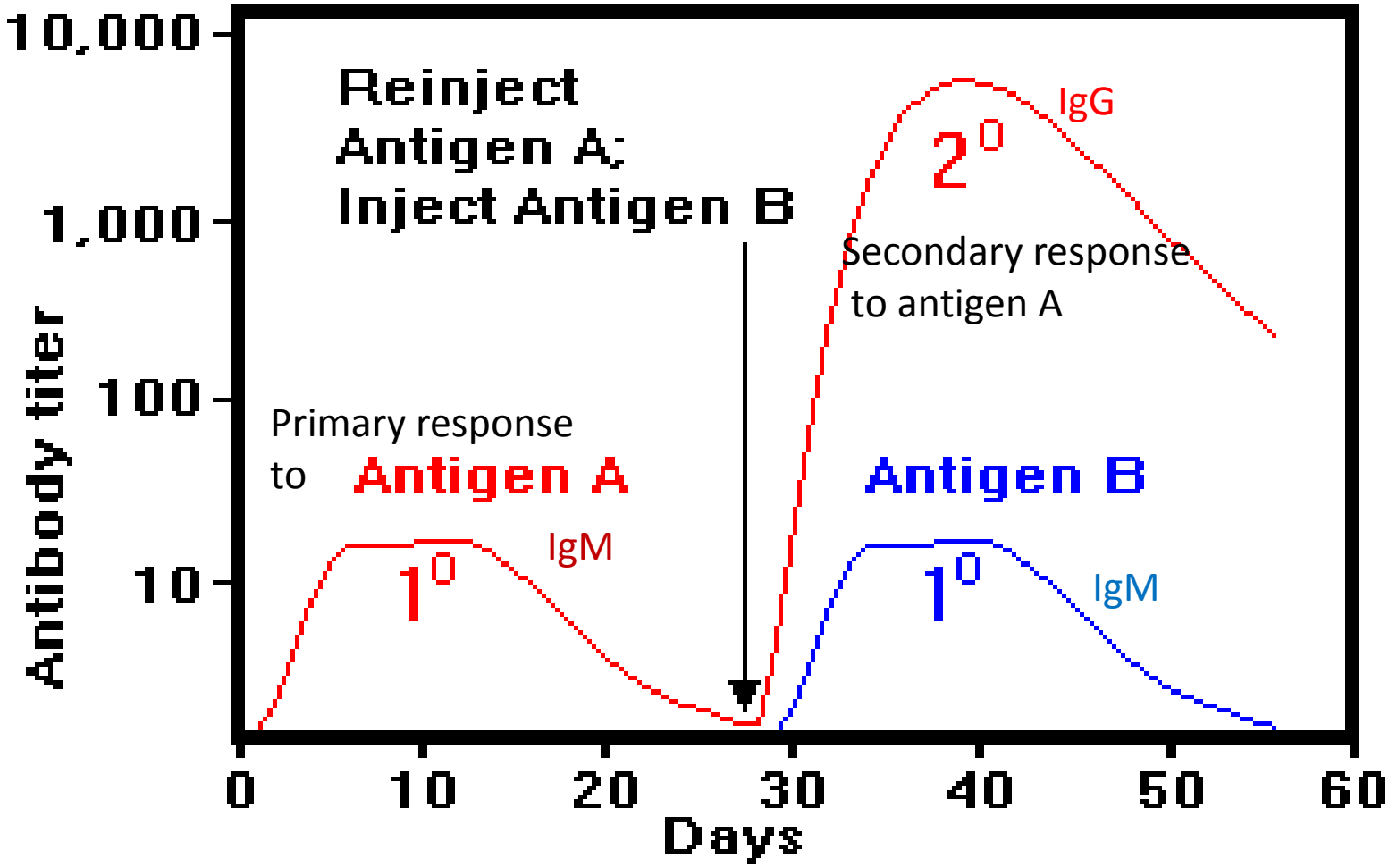
- *In BTS: If primary Imm. Response, no need for another Transfusion (Lysis).*
- *Secondary Immune response:* ***the person encounters an Ag she/he has already encountered and responded to in his/her life. specific Abs are already there***

Primary and Secondary response: Kinetics of antibody production

- Animal injected with Antigen A at day 0. Antigen A invokes a primary response beginning about day 4, as indicated by a rise in the specific antibody titer (titer = measure of the amount of antibody in the animal's serum per unit volume)
- Initial antibody mostly IgM to a peak titer between days 7 and 10 ten decreasing rapidily. If same animal is then reinjected with Antigen A at day 28, the production of antibody begins almost immediately and reaches a level 1000-fold greater that that seen in the primary response.

- But if a second antigen (Antigen B) is also injected at the same time as the reinjection of Antigen A, only a primary response to Antigen B is observed. These results demonstrate that:
  - The immune response is specific.
  - The immune response has memory.

Primary and secondary response; Antibody Kinetics



BTS: Abs produced

Primary Response:

IgM (Anti-D in primary response are likely to be IgM), then IMMEDIATELY replaced by IgG.

Some times Ig A if hyperimmunization (many consecutive stimulations)

- Allo-Abs: e.g: Rhesus system

Immunized: transfusions, pregnancies

During pregnancy: Mother-fetus ABO incompatibility ***can (not always)*** protect before Anti-D production. how???? (see Antigenic competition)

Allo-Abs in BTS:

Most frequent: Anti-D ***(D Ags: highly immunogenic)***

Factors of Immunogenicity:

1. Foreignness
2. Molecular Size
3. Chemical Composition & Heterogeneity
4. Susceptibility to **Antigen** Processing & Presentation
5. Host factors-genotype/MHC

Then anti-K, anti-$Fy^{a,}$ etc

<u>Immune response (immunization) can be suppressed:</u>

e.g: Mother (D-): if contact with D Ags; can get injected with Anti-D (within 3-13 days). this will prevent her Immune system from reacting-<u>No memory.</u>

Blood Transfusion=introducing Ags in an organism.

If new Ags are introduced (if there had been a contact (natural Ags or Abs) or if Allo-Ags: Secondary Immune response or **Immunization**

e.g: Anti-erythrocytes ***allo-immunization***

Consequences:

- *Immediate Intra-vascular hemolysis (IgM involved. Why???)*
- *Extravascular hemolysis (mostly IgG involved. Why?)*

If Allo-Ags:

- Consequences can come later (if first contact, will need second or third contact for a reaction. E.g: HDNF). **E.g: Maternal allo-immunization**
- Among the antigens capable of causing maternal alloimmunization and fetal hemolytic disease, the Rh blood group system is the most common. In particular, the D antigen of the Rh blood group system (Rh D) causes the most cases of severe hemolytic disease.
- To avoid anti-erythrocytes immunization, never D Ags to a D- lady.

Causes of maternal alloimmunization

Blood transfusion
Fetomaternal hemorrhage
- Antepartum
- Intrapartum

Abortion
- Therapeutic
- Spontaneous

Ectopic pregnancy
Abdominal trauma
Obstetric procedures
- Amniocentesis
- Manual removal of the placenta etc

Lymphocyte Function