BLOOD BANK (ABO BLOOD GROUP SYSTEM)

ABO BLOOD GROUP SYSTEM

1. Almost all normal individuals above 3-6 months of age have **"naturally occuring Abs"** to the ABO antigens they lack
   - These "naturally occuring Abs" are termed because they were thought to arise without antigenic stimulation
2. Naturally occuring Abs are **mostly IgM** class-capable of agglutinating saline/low protein suspended RBCs w/o enhancement and may activate complement cascade

IMPORTANCE OF ABO SYSTEM

- Improper knowledge of ABO system leads to **death**
- Transfusion of even a **small volume** of wrong blood type can lead to death

HEMOLYSIS

- If an individual is transfused with an incompatible blood group **destruction of the red blood cells will occur**
- This may result in the death of the recipient

ABO BLOOD GROUPING SYSTEM

- According to the ABO blood typing system there are four different kinds of blood types:
  - A, B, AB or O (null)
- The only blood group system in which antibodies are consistently, predictably, and naturally present in the serum of people who lack the antigen

BLOOD GROUP A

- If you belong to the blood group A, you have **A antigens on the surface** of your RBCs and **B antibodies in your blood plasma**

BLOOD GROUP B

- If you belong to the blood group B, you have **B antigens on the surface** of your RBCs and **A antibodies in your blood plasma**

BLOOD GROUP AB

- If you belong to the blood group AB, you have **both A & B antigens on the surface of your RBCs** but you **do not have A and B antibodies in your blood plasma**

BLOOD GROUP O

- If you belong to the blood group O (null), you have **neither A or B antigens on the surface of your RBCs** but you have **both A and B antibodies in your blood plasma**

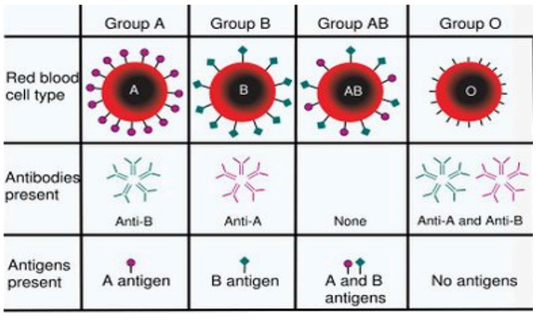
KARL LANDSTEINER (1896-1943)

- In **1900** Karl Landsteiner reported a series of tests, which identified the **ABO Blood Group System**
- In 1910 he won Nobel prize for medicine for this discovery
- He **mixed the serum and cells** of all the researchers in his lab and found four different patterns of agglutination

LANDSTEINER'S RULE FOR ABO GROUP

- A person d**oes not have antibody to his own antigens**
- Each person has antibody to the antigen he lacks (only in the ABO system)

BLOOD GROUP	ANTIGEN/ AGGLUTINOGEN	ANTIBODY/ AGGLUTININ
A	A	anti–B
B	B	anti–A
AB	A,B	NONE
O	NONE	Anti–A, Anti–B, Anti–A,B



ABO TYPING

- Involves both antigen typing and antibody detection

BLOOD BANK (ABO BLOOD GROUP SYSTEM)

- The **antigen typing** is referred to as the **FORWARD TYPING** and the **antibody detection** is the **REVERSE TYPING**

FORWARD TYPING

- Determines **antigens** on patient's or donor's cells

REVERSE TYPING

- Determines **antibodies** in patient's or donor's serum or plasma

Reagents (Forward Typing)

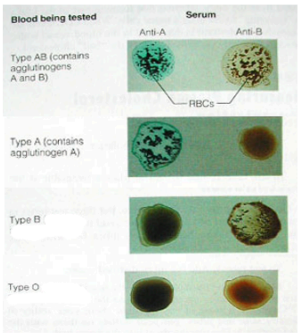
Anti-A Sera

Anti-B Sera

Anti-A,B Sera

REAGENTS

ANTI-A	ANTI-B
Monoclonal antibody	Monoclonal antibody
Highly specific	Highly specific
IgM	IgM
Blue colored **(Patent blue dye)**	Yellow colored **(Acriflavine/Tartrazin)**
Expected 3+ to 4+ rxn	Expected 3+ to 4+ rxn
1-2 drops	1-2 drops



Reagents (Reverse Typing)

Reagent A_1 cells Reagent B cells

REAGENTS

A1 Cells	B Cells
Human Source	Human Source
4-5% Cell Suspension	4-5% Cell Suspension
Expected 2+ to 4+ rxn	Expected 3+ to 4+ rxn
1 drop	1 drop

Forward and Reverse Grouping Reaction

	Reaction of Cells Tested With		Reaction of Serum Tested Against		ABO Group
	Anti-A	Anti-B	A_1 Cells	B Cells	
1	0	0	+	+	O
2	+	0	0	+	A
3	0	+	+	0	B
4	+	+	0	0	AB

DISCREPANCIES IN ABO TYPING

- Results of forward and reverse typing must agree before reporting out blood type
- If forward and reverse do not agree, must identify cause of discrepancy
- **Unresolved discrepancy- report out blood type as UNKNOWN and give Group O blood**

PRINCIPAL TYPES

- A
- B
- AB
- O
- There are two antigens and two antibodies that are mostly responsible for the ABO types – **Antigens A & B**

WHY DO INDIVIDUALS PRODUCE ANTIBODIES TO ANTIGENS THEY DO NOT HAVE?

- The "A", "B", and "H" antigens are also produced by some plants and microorganisms
- Thus, individuals who do not recognize these antigens as "self" will produce antibodies against the plant or microbial antigen and these antibodies will also react with human antigens
- **BACTERIA** – chemically similar to A and B antigens
- **ABO group** – have "Naturally occurring antibodies" to antigens they lack on their red cells
  Example: A: A antigen (RBC) and anti-B (Plasma)

ABO ANTIBODIES

BLOOD BANK (ABO BLOOD GROUP SYSTEM)

- Antibodies are directed against the A & B antigens lacking in the red cells
- Naturally occurring
- Predominantly IgM
- Activates complement
- Cold antibodies
- Small quantities of IgG present

O GROUP ANTIBODIES

- Anti-A, Anti-B, Anti-A,B, and Predominantly IgG

ANTI A, B, IN O

- Reacts with A and B RBCs
- Activities for both RBC groups cannot be separated by adsorption
- Elutes from A RBCs used to adsorb group O serum containing anti-A and anti-A,B antibody that reacts with both A and B RBCs
- Similar findings are obtained when B RBCs are used for adsorption
- Saliva from A or B secretors inhibits the activity of this antibody with either A or B RBCs

ABO ANTIBODIES

- **Initiated at birth** - titer is very low for detection
- Requires 3 to 6 months to increase titer
- **Early at birth** - main source is from mother (IgG)
- Peaks at 5 to 10 years
- **At birth** - only forward typing is performed
- **Geriatrics** - low levels of antibodies (RT not recommended)
- **ABO antigens are glycolipid in nature** - oligosaccharides attached directly to lipids on red cell membrane
- Stick out from red cell membrane and there are many antigen sites per red blood cell (approximately 800,000)
- Moderately well developed at birth
- Therefore ABO-HDN not as severe as other kinds of Hemolytic Disease of the Newborn

ABO GENETICS AND INHERITANCE

- **CHROMOSOME** - unit of inheritance, carries genes, 23 pairs of chromosomes per person, carrying many genes, one chromosome inherited from mother, one from father
- **GENE -** determines specific inherited traits (ex. Blood Type)
- **LOCUS -** site on chromosome where specific gene is located
- **ALLELE -** alternate choice of genes at a locus (ex. A or B; C or c, Lewis a or Lewis b)
- **HOMOZYGOUS** - alleles are the same for any given trait on both chromosomes (ex. A/A)
- **HETEROZYGOUS** - alleles for a given trait are different on each chromosome (ex. A/B or A/O)
- **PHENOTYPE** - observed inherited trait (ex. group A or Rh positive)
- **GENOTYPE** - ◦actual genetic information for a trait carried on each chromosome (ex. O/O or A/O)
- **DOMINANT** - the expressed characteristic on one chromosome takes precedence over the characteristic determined on the other chromosome (ex. A/O types as A)
- **CO-DOMINANT** - the characteristics determined by the genes on both chromosomes are both expressed - neither is dominant over the other (ex. A/B types as AB)
- **RECESSIVE** - the characteristic determined by the allele will only be expressed if the same allele is on the other chromosome also (ex. can type as O only when genotype is O/O)
- The **ABO gene is AUTOSOMAL** (the gene is not on either sex chromosomes)
- The ABO gene locus is located on the **chromosome 9**
- **A and B** blood groups are **dominant** over the O blood group
- **A and B** group genes are **co-dominant**
- Each person has **two copies of genes** coding for their ABO blood group (one maternal and one paternal in origin)
- Family studies have shown that the genes at the remaining two loci, Hh and Sese (secretor), are closely linked.
- The chromosome on which they are located has not yet been identified
- A study has shown H gene is found in Chromosome 19

PHENOTYPE: IS THE VISIBLE PROPERTIES

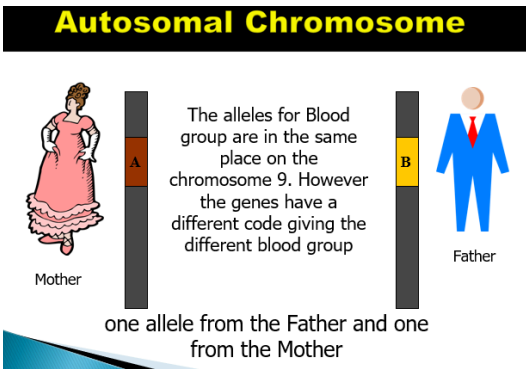
BLOOD BANK (ABO BLOOD GROUP SYSTEM)

OF AN ORGANISMS

GENOTYPE: MAKE UP OF AN ORGANISM

HOMOZYGOUS: HAVING 2 GENES AT CORRESPONDING LOCI/ WHERE AN INDIVIDUAL INHERITS IDENTICAL FORMS OF PARTICULAR GENE FROM EACH PARENT

HETEROZYGOUS: HAVING 2 DIFFERENT VERSIONS OF THAT GENE/ HAVING INHERITED DIFFERENT FORMS OF A PARTICULAR GENE FROM EACH PARENTS



CO-DOMINANT GENES

- If a person **inherited one A group gene** and **one B group gene** their **red cells would possess both the A and B blood group antigens**
- These alleles were termed A (which produced the A antigen), B (which produced the B antigen) and O (which is "non functional" and produced no A or B antigen)

RECESSIVE GENES

- The O gene signifies lack of A or B antigens
- It is not expressed unless this gene is inherited from both parents (OO). Therefore the O gene is recessive

INHERITANCE PATTERNS

- A/A parent can only pass along **A gene**
- A/O parent can pass along either **A or O gene**
- B/B parent can only pass along **B gene**
- B/O parent can pass along either **B or O gene**
- O/O parent can only pass along **O gene**
- AB parent can pass along either **A or B gene**

ABO PHENOTYPE AND GENOTYPE

- Group A phenotype = A/A or A/O genotype
- Group B phenotype = B/B or B/O genotype
- Group O phenotype = O/O genotype
- Group AB phenotype = A/B genotype

PRODUCTION OF A, B, AND H RBC ANTIGEN

- Formation of ABH results from the interaction of genes at three separate loci:
  - ABO
  - Hh
  - Se
- Genes don't code for the production of antigens, rather produce specific transferases
- The production of A, B and H antigens are controlled by the action of transferases
- These transferases are enzymes that catalyze (or control) addition of specific sugars to the oligosaccharide chain
- The H, A, or B genes each produce a different transferase, which adds a different specific sugar to the oligosaccharide chain

TERMS

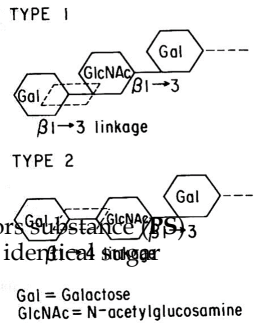
- **Isoagglutinins –** are antibodies that agglutinate blood cells of some individuals of the same species
- **Glycosyltransferases –** are enzymes that facilitate the transfer of carbohydrate (sugar) molecules onto carbohydrate precursor molecules
- **Immunodominant sugar –** is the sugar molecule that completes the antigenic determinant when combined with the precursor substance

PARAGLOBOSIDE

- Basic Precursor substance
- Precursor substance is the TYPE 2 precursor
- Type 2 precursor chain- terminal galactose on the precursor substance is attached to the **N-acetylglucosamine in a beta 1-4 linkage**

PRECURSOR SUBSTANCE

- 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



ABH ANTIGENS

- Two potential precursors substance (PS) both are comprised of identical sugar

(**galactose-N-acetyl glucosamine-galactose-glucose**) but different in linkage

- **Type I PS** has a terminal galactose (Gal) linked to a subterminal N-acetylglucosamine (GlcNAc) in **1-3** linkage
- **Type II PS** has the same sugar combine in **1-4** linkage

- ABH Antigens on RBCs are derived from **Type II chains**

- A Locus termed H and the final product of the genes at that locus, H antigen, is necessary for the expression of normal ABO antigens

- ABH Antigens of the RBC membrane are found partly as glycolipids, but primarily as glycoproteins. It may also occur in the secretion as glycoproteins

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

- The presence of A,B, and O Antigens on RBCs depends upon the allelic genes: A,B, and O

- An H genes 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 a specific transferase**s**

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- **H** gene products is an enzyme that produce **H substance**

- α-2-L-fucosyltransferase w/c transfers **L-fucose** to an oligosaccharide chain on the terminal galactose

- The **O gene is a silent allele**

- It does not alter the structure of **H** substance

ABO GENETICS

- ***Se* genes – *Se* and *se* alleles**

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

  - *se* allele is an amorph

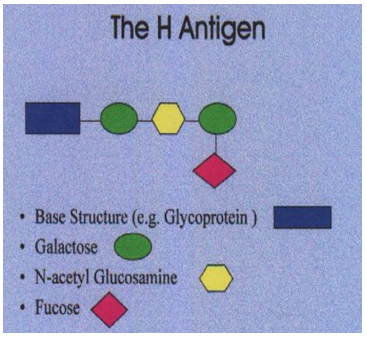
- ***ABO* genes – *A*, *B* and *O* alleles**

  - *A* and *B* alleles code for **glycosyltransferase**, a fucosyltransferase enzyme that adds a sugar into H antigens to produce A and B antigens

  - *O* allele does not code a functional enzyme

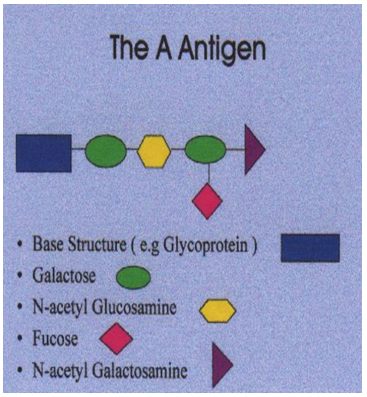
H ANTIGENS

- H gene codes for a **fucosyl transferase (FT)**
- FT causes L-fucose to be added to the terminal sugar of precursor chain, producing H antigen-Type 2 H antigen saccharide chain



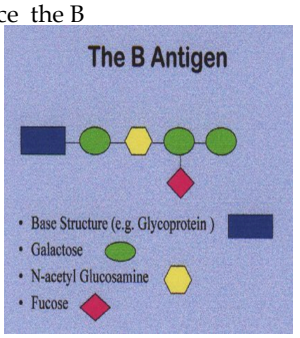
A ANTIGENS

- **produced an enzyme *glycosyl transferase* (GT)**
- **(GT) uses the H antigen as a substrate and catalyses the addition of the sugar *N acetyl galactosamine* to the terminal *galactose* to produce the A antigen**
- **The A enzyme can only produce the A antigen if a H antigen is already present**



B ANTIGENS

- produced by an enzyme glycosyl transferase (GT)
- GT uses the H antigen as a substrate and catalyzes the addition of the sugar galactose to the terminal galactose of the H antigen
- The B enzyme can ONLY produce the B antigen if a H antigen is present



AMOUNT OF H ANTIGEN

BLOOD BANK (ABO BLOOD GROUP SYSTEM)

Blood Group O people have rbc 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**

Donor Nucleotides & Immunodominant Sugars Responsible for H, A, B Antigen Specificity

Antigen	Immunodominant sugar	Glucosyltransferase	Gene
H	L-fucose	L- fucosyl transferase	H
A	N-acetyl-D-galactosamine	N-acetylgalactosaminyl transferase	A
B	D-galactose	D- galactosyl transferase	B

ABO ANTIGENS

- If both A and B genes present, some H-chains converted to A antigen, some converted to B antigen
- If H gene is absent (extremely rare), no H substance can be formed, and therefore no A or B antigen. Result is **BOMBAY BLOOD GROUP**

BOMBAY BLOOD GROUP

- **lacks H gene** – cannot make H antigen (H substance)
- Since the H substance is the precursor for the A and B antigens, these antigens also are not made
- The cells type as **O** and the serum has **anti-A**, **anti-B**, and **anti-H** since the individual lacks all of these antigens
- **Anti-H** agglutinates **O cells**
- The only cells Bombay individuals do not agglutinate are from other Bombay blood people since they lack the H antigen

BOMBAY BLOOD GROUP

O		BOMBAY	
ANTIGEN	ANTIBODY	ANTIGEN	ANTIBODY
NONE	Anti–A	NONE	Anti–A
NONE	Anti–B	NONE	Anti–B
H	NONE	NONE	Anti–H

SUBGROUPS OF A AND B

- The subgroups of A and B are caused by decreased amounts of antigen on the red blood cells
- They are inherited conditions
- The most common are subgroups of A
- Approximately 80% of the A's and AB's have a normal expression of **A1**
- Most of the other 20% are either **A2** or **A2B** – this subgroup has fewer H chains converted to A antigen

LECTINS

- extracts of seeds of plants that react specifically with certain antigens
- The common lectins used in Blood Bank are:

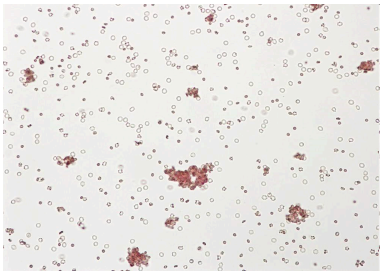
- *Ulex europaeus*, or **lectin H**, which agglutinates cells that have H substance

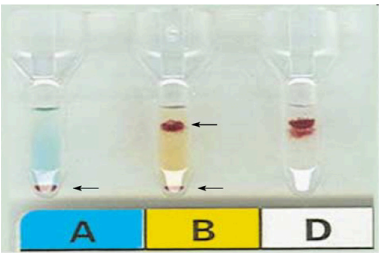
- *Dolichos biflouros*, or **lectin A1**, which agglutinates cells with A1

- *Bandeiraea simplicifolia*, or **lectin B**, which agglutinates B cells

A1 VS. A2

- **A1 phenotype** – reacts with **anti-A1** and make up about 80% of blood type A
- **A2 phenotype – do not** react with **anti-A1** and are about 20% of blood type A
- A1 red cells express about 5 times more A antigen than A2 red cells
- Both react with anti-A as far as transfusion purposes are concerned, the A1 and A2 blood groups are interchangeable.
- Use lectin-A1 to differentiate A1 cells from all others - will agglutinate only A1 cells
- Look for weaker or mixed field reactions
- Look at strength of reactions with anti-A,B or with lectin-H





Monoclonal gel card demonstrating the mixed field agglutination (arrows). A mixed field agglutination pattern was observed with anti-B indicating the simultaneous presence of two red blood cell populations.