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ABO Blood group

ABO blood group

- The ABO blood group system was discovered by **Landsteiner in 1901.**
- ABO blood group phenotypes include **A, B, AB, and O.**
- The frequency of the four **ABO groups varies in different populations**: Amerindians are almost exclusively group O while Asians have a higher incidence of group B.
- Individuals form antibodies **(anti-A and anti-B)** to the **antigens they lack.** These antibodies are termed **"naturally occurring,"** as they are present in sera of individuals without previous red blood cell (RBC) exposure. In some cases, the **antibodies have already been induced by natural exposure to similar antigenic determinants on a variety of microorganisms present in the normal flora of the gut.**
- Antibodies to the A, B, and O antigens, called isohemagglutinins, are usually of the **IgM class.** These IgM antibodies **can activate complement and can thus cause severe intravascular hemolysis (complement mediated lysis) after transfusion of ABO-incompatible blood components**, making them the most clinically significant antibodies in transfusion practice.

Antigens of the ABO blood group

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- **Number of antigens:** A (A1 and A2), B, AB.
- **Antigen specificity:** Carbohydrate (the **sequence of oligosaccharides** determines whether the antigen is A or B).
- **Antigen-carrying molecules:** Glycoproteins and glycolipids (the ABO blood group **antigens are attached to oligosaccharide chains that project above the RBC surface.** These chains are attached to proteins and lipids that lie in the RBC membrane).
- **Molecular basis:** The ABO gene indirectly encodes the ABO blood group antigens (the ABO locus has **three main allelic forms: A, B, and O**. The **A and B** alleles each **encode a glycosyltransferase** that catalyzes the final step in the synthesis of the A and B antigen, respectively. The **O allele encodes an inactive glycosyltransferase** that leaves the ABO antigen precursor or the **H antigen unmodified.**

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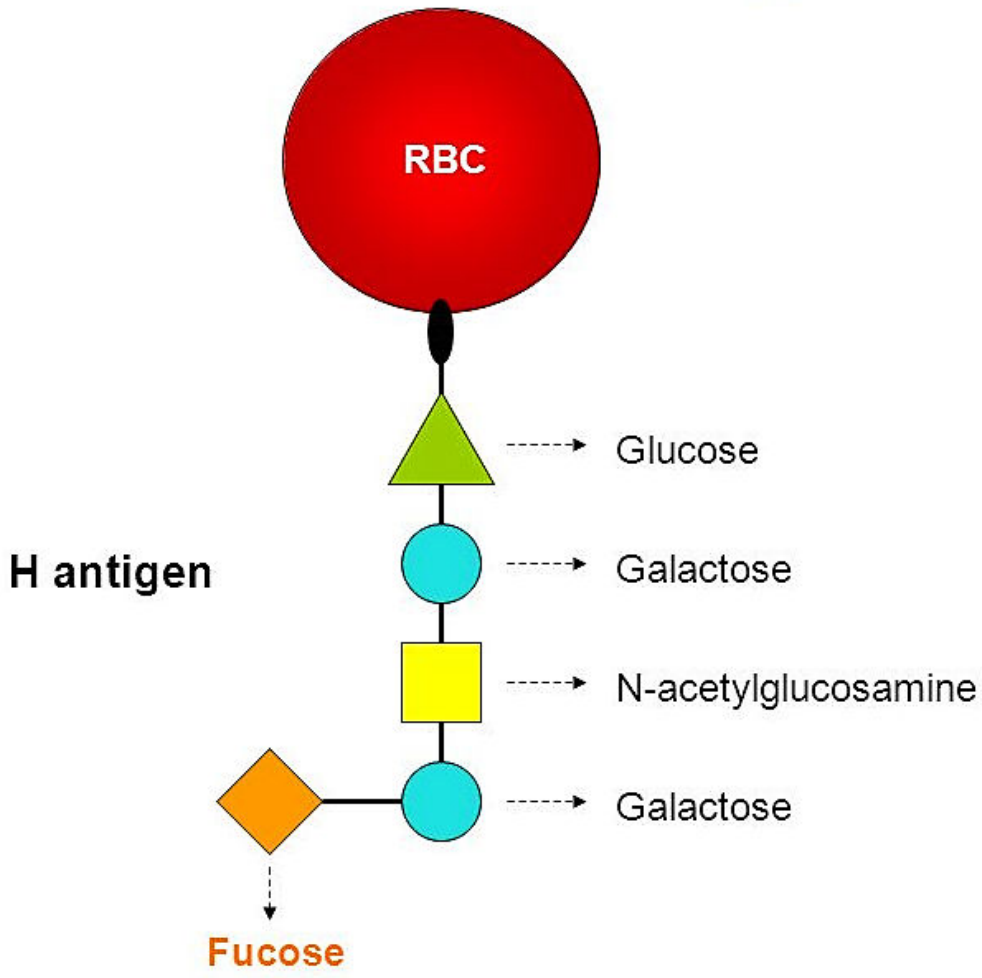
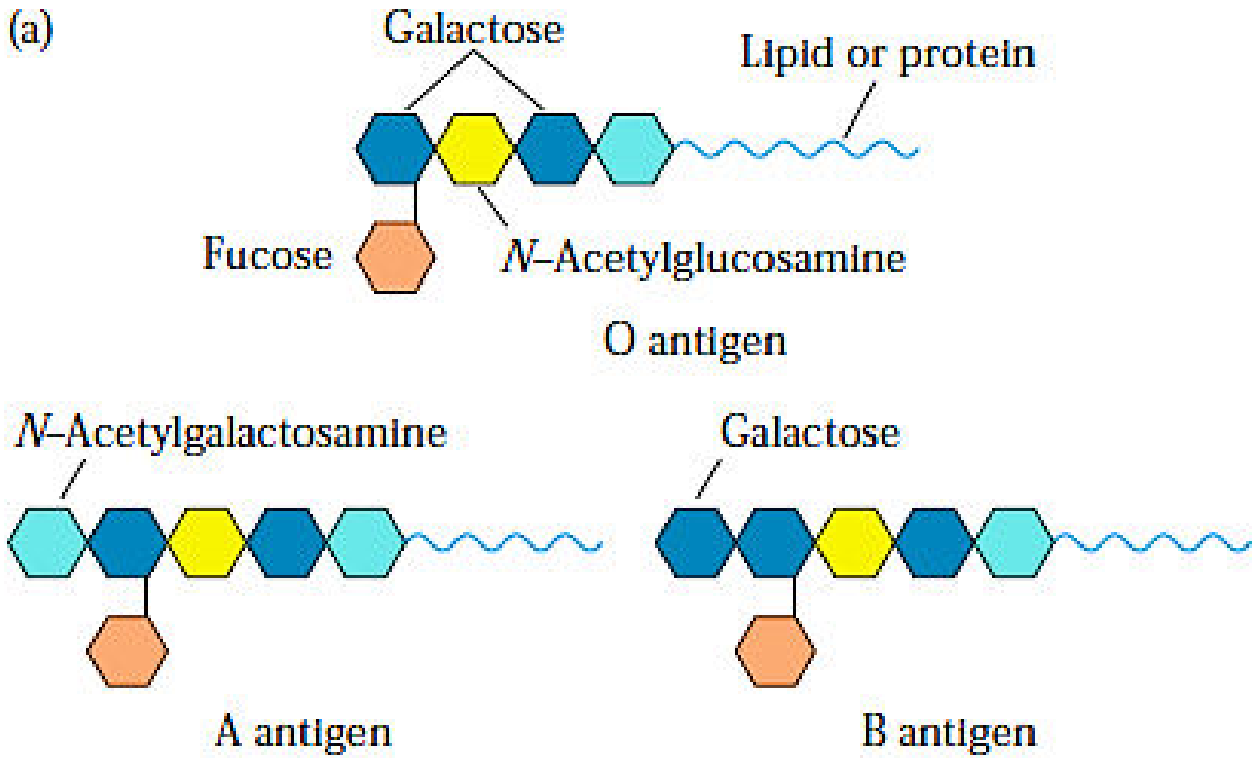
H antigen (O blood group)



Alleles for antigens and modifications of H antigens by glycosyltransferase

- The ABO locus has **three main alleleic forms**: A, B, and O.
- The **A allele encodes a glycosyltransferase** that produces the A antigen (**N-acetylgalactosamine** is its immunodominant sugar), and the **B allele encodes a glycosyltransferase** that creates the B antigen (**D-galactose** is its immunodominant sugar).
- The **O allele** encodes an enzyme with no function, and therefore **neither A or B antigen is produced,** leaving the underlying precursor (the H antigen) **unchanged.**
- The A and B antigens differ from the O antigen by the **addition of one extra monosaccharide, either *N*-acetylgalactosamine (for A) or galactose (for B)** through an a **-1,3 linkage** to a galactose moiety of the O antigen.
- **Specific glycosyltransferases add the extra monosaccharide to the O antigen**. Each person **inherits the gene** for one glycosyltransferase of this type from each parent. The **type A transferase specifically adds N-acetylgalactosamine**, whereas the **type B transferase adds galactose.**

Structure of terminal sugars, which constitute the distinguishing epitopes, in the A, B, and O blood antigens



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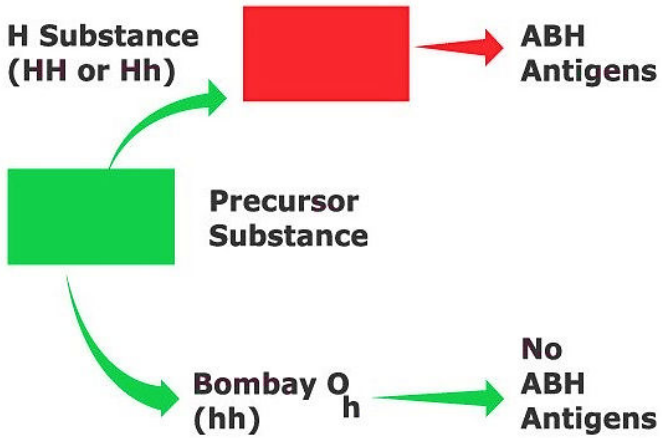
Bombay phenotype

- Named for the city in which it was first discovered, the "Bombay phenotype" describes individuals whose RBCs lack the H antigen.
- Because the A and B antigens cannot be formed without the H antigen precursor, their RBCs also lack these antigens. **H antigen deficiency is known as the "Bombay phenotype" (h/h, also known as Oh)** and is found in 1 of 10,000 individuals in India and 1 in a million people in Europe.
- As a result, these **individuals produce anti-H, anti-A, and anti-B** and can therefore be transfused only with RBCs that also lacks the H, A, and B antigens i.e., **they can only receive blood from another person with the Bombay phenotype.**
- Because of the rarity of this blood type, this normally means using blood donations from a suitable relative.



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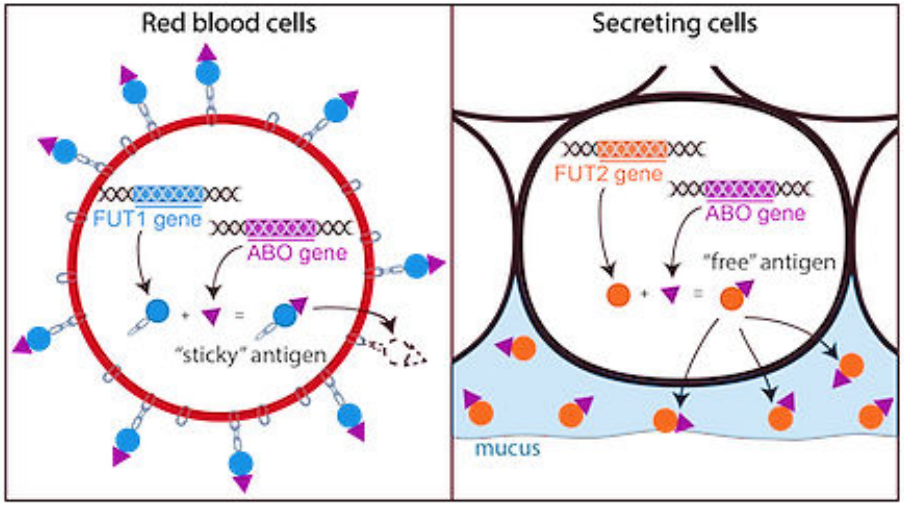
Secretors and non-secretors concept

- Human population can be categorized into **secretors and non-secretors.**
- They are categorized on the **basis of presence or absence of the blood group antigens (A, B and H) in the body fluids and secretions, such as saliva, sweat, tears, semen, serum, mucus present in the digestive tract or respiratory cavities etc.**
- **Secretors** are individuals that **secrete blood group antigens in their body fluids** while **non-secretors** are the individuals that **do not secrete them in their body fluids and secretions.**
- It is a known fact that ABO blood type is controlled by blood type coding genes present on the chromosome 9q34 but the secretor status of an individual is decided by interaction of a separate gene (called secreting gene) with these blood type genes.
- The presence of the secreting gene in a person's genome makes him a secretor and absence makes him a non secretor.
- The **gene is designated as (Se) for Secretors and (se) for Non-secretors and it is entirely independent of the blood type A, B, AB or O.**
- The individuals secreting antigens in the body fluid are designated as 'ABH secretors' in blood banks.
- Individuals having O blood group secrete antigen H, A blood group secrete A and H antigens, B blood group secrete B and H antigens in the fluids.



ABO phenotypes

- The four basic ABO phenotypes are **O, A, B, and AB.**
- After it was found that blood group A RBCs reacted differently to a particular antibody (later called anti-A1), the blood group was divided into two phenotypes, **A_1 and A_2.**
- RBCs with the **A_1 phenotype** react with anti-A1 and make up about **80%** of blood type A.
- RBCs with the **A_2 phenotype** do not react with anti-A1 and they make up about **20%** of blood type A.
- There are **many other subgroups of blood group A** in which RBCs tend to weakly express the A antigen, whereas **weak variants of the blood group B phenotype** are rare.
- Thus, a **group A individual will have A antigen and anti-B antibodies** and a **group B individual will have B antibody and anti-A antibodies.**
- Blood group **O is common**, and individuals with this blood type will have **both anti-A and anti-B in their serum but absence of both A and B antigens.**
- Blood group **AB is the least common,** and these individuals will have **neither anti-A nor anti-B in their serum but presence of both A and B antibodies.**

ABO blood group system

Antigen (agglutinogen) and antibody (agglutinin) in ABO blood subgroup system

Blood group		Antigen on the RBC	Antibody in the serum
A	A1	A+ A1	Anti-B
	A2	A	Anti-B+ Anti-A1
B		B	Anti-A
AB	A1B	A+ A1 +B	—
	A2B	A+B	Anti-A1
O		—	Anti-A+Anti-B

Difference between A1 and A2 subgroups

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A2 Phenotype Has Lower Cell Surface Expression of the A Antigen on Red Blood Cells

- It's quantitative.
- The A2 gene doesn't convert the H to A very well – The result is fewer A2 antigen sites compared to the many A antigen sites.
- There are other additional subgroups of A – A_{int} (intermediate), A_3, A_x, Am, A_{end}, A_{el}, A_{bantu}.

Differentiating Subgroups of A

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Use the following steps to help differentiate the subgroups of A:

- Use lectin-A_1 to differentiate A_1 cells from all others - will agglutinate only A_1 cells
- Look for weaker or mixed field reactions
- Look for anti-A_1 in serum (serum reacts with A_1 cells but not A_2 cells)
- Look at strength of reactions with anti-A,B or with lectin-H.

	Anti-A antisera	Anti-A_1 antisera	Anti-H lectin	ABO antibodies in serum	# of antigen sites per RBC
A_1	4+	4+	0	Anti-B	900 x10^3
A_2	4+	0	3+	Anti-B & anti-A_1	250 x10^3

Lectins

Lectins are extracts of seeds of plants that react specifically with certain antigens. The two most 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.

Lectin-H reacts strongest with O cells, which has a high concentration of H antigen, and weakest with A1 cells, which have a low concentration of H.

ABO genotypes and inheritance pattern

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Blood Type	Genotype		Can Receive Blood From:
A	$i^A i$ $i^A i^A$	AA AO	A or O
B	$i^B i$ $i^B i^B$	BB BO	B or O
AB	$i^A i^B$	AB	A, B, AB, O
O	$i\,i$	OO	O

		O	A		B		AB
	Alleles	ii (OO)	I^Ai (AO)	I^AI^A (AA)	I^Bi (BO)	I^BI^B (BB)	I^AI^B (AB)
O	ii (OO)	**O** OO OO OO OO	**O or A** AO OO AO OO	**A** AO AO AO AO	**O or B** BO OO BO OO	**B** BO BO BO BO	**A or B** AO BO AO BO
A	I^Ai (AO)	**O or A** AO AO OO OO	**O or A** AA AO AO OO	**A** AA AA AO AO	**O, A, B or AB** AB AO BO OO	**B or AB** AB AB BO BO	**A, B or AB** AA AB AO BO
	I^AI^A (AA)	**A** AO AO AO AO	**A** AA AO AA AO	**A** AA AA AA AA	**A or AB** AB AO AB AO	**AB** AB AB AB AB	**A or AB** AA AB AA AB
B	I^Bi (BO)	**O or B** BO BO OO OO	**O, A, B or AB** AB BO AO OO	**A or AB** AB AB AO AO	**O or B** BB BO BO OO	**B** BB BB BO BO	**A, B or AB** AB BB AO BO
	I^BI^B (BB)	**B** BO BO BO BO	**B or AB** AB BO AB BO	**AB** AB AB AB AB	**B** BB BO BB BO	**B** BB BB BB BB	**B or AB** AB BB AB BB
AB	I^AI^B (AB)	**A or B** AO AO BO BO	**A, B or AB** AA AO AB BO	**A or AB** AA AA AB AB	**A, B or AB** AB AO BB BO	**B or AB** AB AB BB BB	**A, B or AB** AA AB AB BB

Antibodies produced against ABO blood group antigens

Antibody type	**IgG and IgM** Naturally occurring. (There is some evidence that similar antigens found in certain bacteria, like ***E.coli***, stimulate antibody production in individuals who lack the specific A and B antigens). Anti-A is found in the serum of people with blood groups O and B. Anti-B is found in the serum of people with blood groups O and A.
Antibody reactivity	**Capable of hemolysis** Anti-A and anti-B bind to RBCs and activate the complement cascade, which lyses the RBCs while they are still in the circulation (intravascular hemolysis).
Transfusion reaction	**Yes** — typically causes an acute hemolytic transfusion reaction. Most deaths caused by blood transfusion are the result of transfusing ABO-incompatible blood.
Hemolytic disease of the newborn	**No or mild disease** HDN may occur if a group O mother has more than one pregnancy with a child with blood group A, B, or AB. Most cases are mild and do not require treatment.

Compatible blood transfusion

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Blood Type	Gives	Receives
A+	A+, AB+	A+, A-, O+, O-
O+	O+, A+, B+, AB+	O+, O-
B+	B+, AB+	B+, B-, O+, O-
AB+	AB+	Everyone
A-	A+, A-, AB+, AB-	A-, O-
O-	Everyone	O-
B-	B+, B-, AB+, AB-	B-, O-
AB-	AB+, AB-	AB-, A-, B-, O-

How to read your results?

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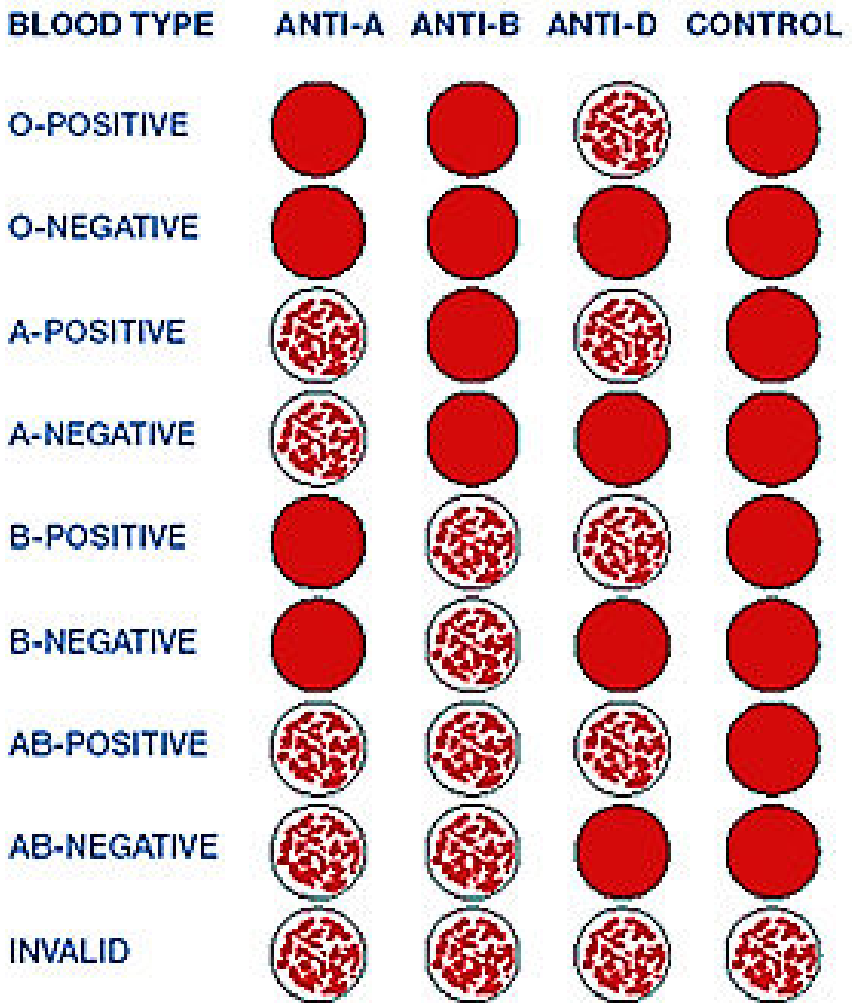

Results of Incompatible Blood Transfusion

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Immediate

- Reactions lead to **complement mediated lysis** triggered by the IgM isohemagglutinins.
- Within hours, **free hemoglobin can be detected in** the plasma; it is filtered through the kidneys, resulting in hemoglobinuria.
- Some of the **hemoglobin gets converted to bilirubin**, which at high levels is **toxic.**
- Typical symptoms include **fever, chills, nausea, clotting within blood vessels, pain in the lower back, and hemoglobin in the urine.**
- Treatment involves prompt termination of the transfusion and maintenance of urine **flow with a diuretic, because the accumulation of hemoglobin in the kidney can cause acute tubular necrosis.**

Delayed

- Delayed hemolytic transfusion reactions generally occur in individuals who have received **repeated transfusions** of ABO-compatible blood that is incompatible for other blood group antigens.
- The reactions develop between **2 and 6 days after transfusion**, reflecting the secondary nature of these reactions.
- The transfused blood **induces clonal selection and production of IgG against a variety of blood-group membrane antigens, most commonly Rh, Kidd, Kell and Duffy.**
- The **predominant isotype involved in these reactions is IgG**, which is less effective than IgM in activating complement.
- For this reason, **complement-mediated lysis of the transfused red blood cells is incomplete**, and many of the **transfused cells are destroyed at extravascular sites by agglutination, opsonization, and subsequent phagocytosis by macrophages.**
- Symptoms include **fever, low hemoglobin, increased bilirubin, mild jaundice, and anemia.**
- **Free hemoglobin is usually not detected in the plasma or urine** in these reactions because **RBC destruction occurs in extravascular sites.**

Universal donor and recipient

- Donors with type **O^- red blood cells are referred to as universal donors** and their red blood cells can be given to any other blood type. type O negative blood is compatible to any blood recipient's type. Ideally the donor's blood types should always be an exact match to the recipient's blood type. **Universal donors should only be used in the case of medical emergency** where there is an unavailability or shortage of the patient's blood type or when a blood transfusion needs to be performed immediately, not allowing the time to cross type the blood compatibility.
- Donors with type **AB^+ are referred to as universal recipients** and can receive red blood cells from any other blood type. The reason is that AB blood types **do not contain natural antibodies against the ABO blood groups** and this avoids incompatibility reactions when a person who is blood group AB receives blood from a donor who has another ABO blood group. Blood group AB is very rare and although AB blood types are able to receive any type of blood they are **not able to donate blood to persons that are not blood group AB.**

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Rh factor

Rh factor

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- The **Rhesus factor, or Rh factor**, is a certain type of protein found on the outside of red blood cells. This protein is also often called the **D antigen**.
- It has become **second in importance only** to the ABO blood group in the field of transfusion medicine. It has remained of primary importance in obstetrics, being the **main cause of hemolytic disease of the newborn (HDN).**
- Philip Levine, in 1939, discovered that the sera of most women who gave birth to infants with hemolytic disease contained an antibody that reacted with the red cells of the infant and with the red cells of 85% of Caucasians.
- In **1940, Landsteiner and Wiener** injected blood from the **monkey *Macacus rhesus*** into rabbits and guinea pigs, and discovered the resulting antibody agglutinated rhesus (Rh) red cells, which appeared to have the same specificity as the neonatal antibody.
- The terms Rh factor and Rh antigen are similar, and both refer to the **RhD antigen only**.
- Of all the Rh antigens (C, c, D, E, and e) , **antigen D (RhD) is most important.** Rh antigens are highly **immunogenic, in order of decreasing immunogenicity D > c > E > C > e.**
- People are either **Rh-positive/"RhD positive" (**they have the protein) or **Rh-negative/"RhD negative"** (they do not have the protein).
- "RhD positive" or "RhD negative" is indicated as a suffix to the ABO blood group. This suffix is often shortened to **"D pos"/"D neg," "RhD pos"/"RhD neg," or +/-.**
- RBCs **lacking all Rh antigens** are uncommon and designated as **Rh_{null}.**
- Unlike the ABO system, there are **no natural antibodies against Rh antigens**. Antibodies against Rh antigens develop only in certain situations, such as in **Rh incompatible pregnancy or transfusion.** Most of these antibodies are **IgG antibodies and few IgM antibodies.**
- The **protein is genetically inherited** (passed down from your parents). If you have the protein, you are Rh-positive. If you did not inherit the protein, you are Rh-negative. The majority of people, about **85%, are Rh-positive.**

RH genes on chromosome 1

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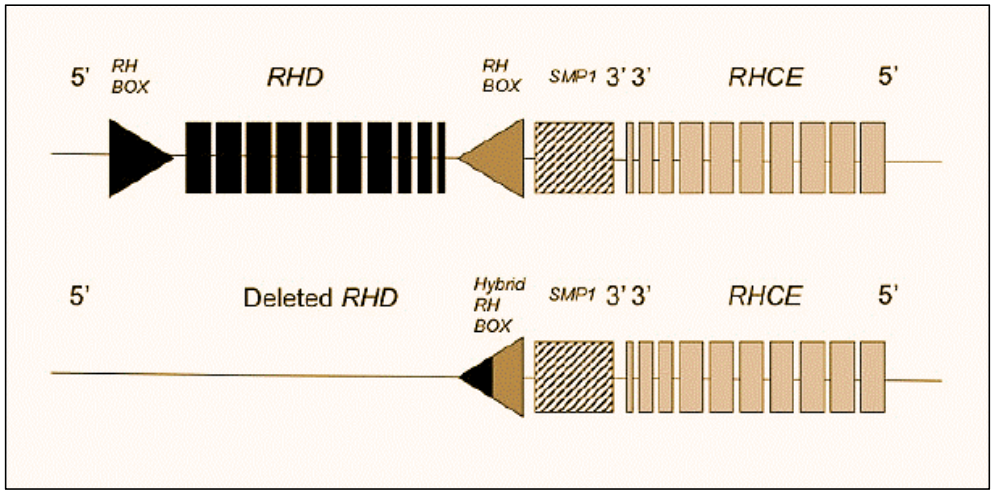

- The complexity of the Rh blood group antigens begins with the highly polymorphic genes that encode them.
- There are two genes, RHD and RHCE, that are closely linked.
- Numerous genetic rearrangements between them has produced hybrid Rh genes that encode a myriad of distinct Rh antigens. To date, 49 Rh antigens are known.

- In the upper panel the gene organization is given as found in D-positive individuals.
- Notice the inverted orientation of the RHD and RHCE genes. In the lower panel the RHD gene deletion is shown after an unequal cross-over between the upstream and downstream RH BOXES, which is the main reason for the D-negative.
- The RhD and RhCE proteins are both transmembrane, multipass proteins that are integral to the RBC membrane. The RhCE protein encodes the C/c antigen (in the 2nd extracellular loop) and the E/e antigen (in the 4th extracellular loop), plus many other Rh antigens e.g., C^w, C^x.
- Unlike most cell surface molecules, the Rh proteins are not glycosylated (they do not contain oligosaccharides) but they are closely associated with a RBC membrane glycoprotein called RhAG. The function of the Rh-RhAG complex might involve transporting ammonium or carbon dioxide. The Rh antigens are thought to play a role in maintaining the integrity of the RBC membrane—RBCs which lack Rh antigens have an abnormal shape.

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Antibodies produced against Rh antigens

Antibody type	**Mainly IgG, some IgM** The majority of Rh antibodies are of the IgG type.
Antibody reactivity	**Capable of hemolysis** Rh antibodies rarely activate complement. They bind to RBCs and mark them up for destruction in the spleen (extravascular hemolysis).
Transfusion reaction	**Yes—typically delayed hemolytic transfusion reactions** Anti-D, anti-C, anti-e, and anti-c can cause severe hemolytic transfusion reactions. Hemolysis is typically extravascular.
Hemolytic disease of the newborn	**Yes—the most common cause of HDN** The D antigen accounts for 50% of maternal alloimmunization. Anti-D and anti-c can cause severe disease. Anti-C, anti-E, and anti-e can cause mild to moderate disease.

Rh negative mother and Rh positive child

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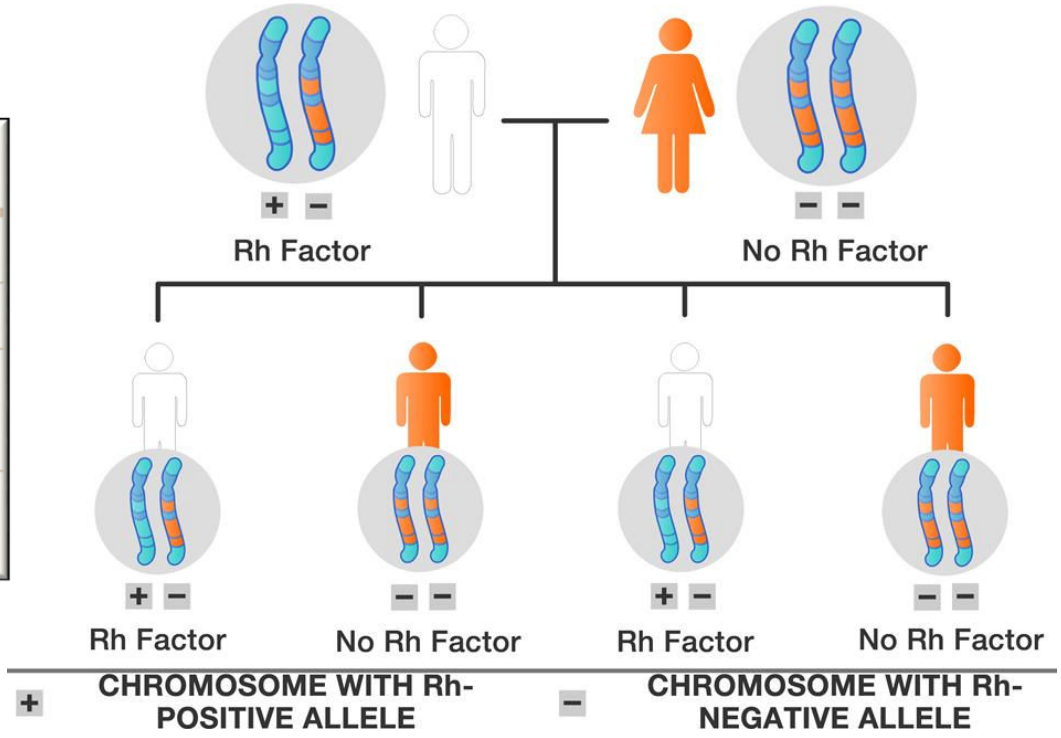
Mother's Rh factor	Father's Rh factor	Baby's Rh factor	Precautions
Rh positive	Rh positive	Rh positive	None
Rh negative	Rh negative	Rh negative	None
Rh positive	Rh negative	Could be Rh positive or Rh negative	None
Rh negative	Rh positive	Could be Rh positive or Rh negative	Rh immune globulin injections



Rh^- mother is exposed to Rh^+ cells

- Within 30 days of exposure, the mother will develop anti-Rh antibodies.
- However, these large (900,000 MW) **IgM antibodies cannot cross the placenta.** Therefore, **the first child will be unaffected.**
- If the **mother is exposed to Rh T cells during a second pregnancy, small (150,000 MW) IgG antibodies are produced.** These antibodies can cross the placenta and attack the fetal red blood cells, causing an autoimmune hemolytic anemia, called **erythroblastosis fetalis, with severe consequences.**
- The **lysis of red cells liberates hemoglobin**, which is converted to bilirubin. **Accumulation of bilirubin damages the central nervous system, and the infants develop hypotonia, hearing loss, and intellectual disabilities.**
- Severe forms of erythroblastosis fetalis are characterized by **cardiac failure, pericardial effusions, and edema (hydrops fetalis).**

Erythroblastosis fetalis and Rhogam

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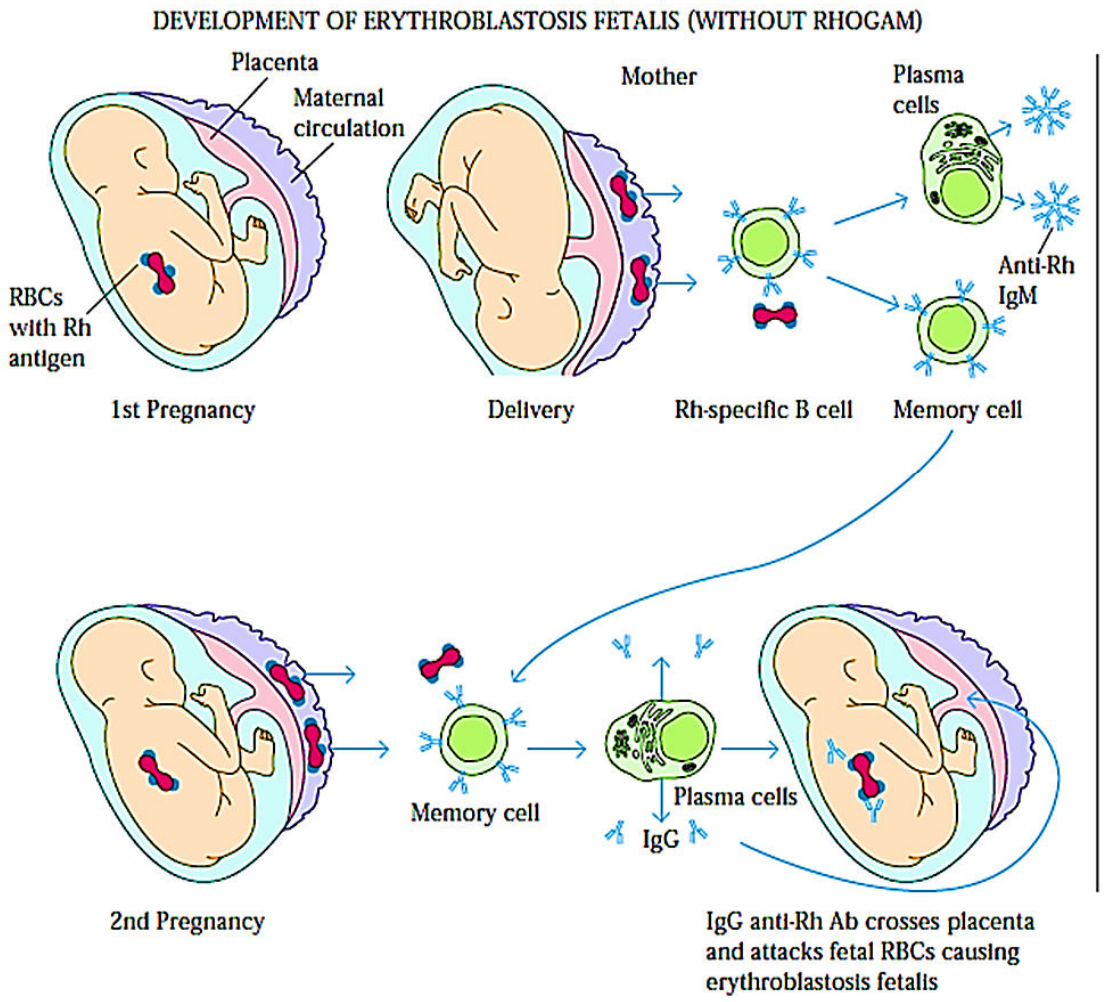

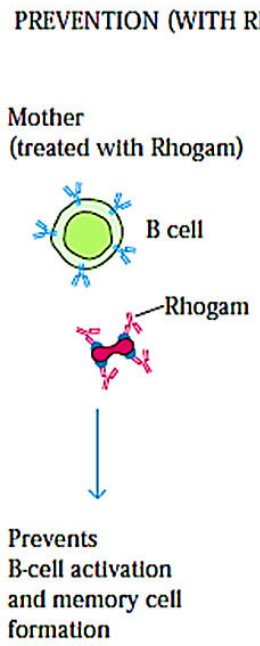

RhoGAM is an **injection made up of antibodies called immunoglobulin** that help protect a fetus from its mother's antibodies

Within **24–48 h** after the first delivery.

RhoGAM **binds to any fetal red blood cells** that enter the mother's circulation at the time of delivery and **facilitate their clearance before B-cell activation and ensuing memory-cell production can take place.**